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XENCOR EXHIBIT 1061 (Part 1 of 3)
Xencor, Inc. v. Merus N.V.
IPR2025-00605

PTO/AIA/15 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	067461-5161-US06
First Named Inventor	John R. Desjarlais
Title	NOVEL HETERODIMERIC PROTEINS
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☐ **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. ☐ **Applicant asserts small entity status.** See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☑ **Specification** [*Total Pages* 81] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. ☑ **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 104]
6. **Inventor's Oath or Declaration** [*Total Pages* 4] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. ☐ Newly executed (original or copy)
   b. ☑ A copy from a prior application (37 CFR 1.63(d))
7. ☑ **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   ☐ Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. ☑ Computer Readable Form (CRF)
   b. ☐ Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper
   c. ☐ Statements verifying identity of above copies

ADDRESS TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers** (cover sheet & document(s)) Name of Assignee _____
11. ☐ **37 CFR 3.73(c) Statement** ☐ **Power of Attorney** (*when there is an assignee*)
12. ☐ **English Translation Document** (*if applicable*)
13. ☐ **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    ☐ Copies of citations attached
14. ☐ **Preliminary Amendment**
15. ☐ **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. ☐ **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. ☐ **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☐ **Other:** _____

*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

☑ The address associated with Customer Number: 67374 **OR** ☐ Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Louis T. Nguyen/	Date	June 26, 2017
Name (Print/Type)	Louis T. Nguyen	Registration No. (Attorney/Agent)	58,729

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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

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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

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
	John		Desjarlais	

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

City	Pasadena	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Xencor, Inc.
Address 2	111 West Lemon Avenue

City	Monrovia	State/Province	CA
Postal Code	91016	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Gregory		Moore	

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

City	Azusa	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Xencor, Inc.
Address 2	111 West Lemon Avenue

City	Monrovia	State/Province	CA
Postal Code	91016	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Rumana		Rashid	

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

City	Temple City	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Xencor, Inc.
Address 2	111 West Lemon Avenue

City	Monrovia	State/Province	CA
Postal Code	91016	Country	US

Inventor 4 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Matthew		Bernett	

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

City	Monrovia	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Xencor, Inc.
Address 2	111 West Lemon Avenue

City	Monrovia	State/Province	CA
Postal Code	91016	Country	US

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

Correspondence Information:

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

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

Customer Number	67374		
Email Address	sfipdocketing@morganlewis.com	Add Email	Remove Email

Application Information:

Title of the Invention	NOVEL HETERODIMERIC PROTEINS		
Attorney Docket Number	067461-5161-US06	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	104	Suggested Figure for Publication (if any)	

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	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

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	67374		

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	14155344	2014-01-14

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14155344	Claims benefit of provisional	61780310	2013-03-13
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14155344	Claims benefit of provisional	61778148	2013-03-12
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14155344	Claims benefit of provisional	61764954	2013-02-14
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14155344	Claims benefit of provisional	61752347	2013-01-14
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.			Add

Foreign Priority Information:

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

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.			Add

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

☐ 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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

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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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

Applicant Information:

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

Applicant 1 Remove

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

Clear

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

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

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Xencor, Inc.		
Mailing Address Information For Applicant:			
Address 1	111 West Lemon Avenue		
Address 2			
City	Monrovia	**State/Province**	CA
Country	US	Postal Code	91016
Phone Number		Fax Number	
Email Address			

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

Assignee Information including Non-Applicant Assignee Information:

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

Assignee 1

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

Remove

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

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 i		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. Add

Signature:

Remove

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	/Louis T. Nguyen/			Date (YYYY-MM-DD)	2017-06-26
First Name	Louis	Last Name	Nguyen	Registration Number	58729

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

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	067461-5161-US06
	Application Number	
Title of Invention	NOVEL HETERODIMERIC PROTEINS	

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Privacy Act Statement

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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.

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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.

NOVEL HETERODIMERIC PROTEINS

PRIORITY

[0001] This application is a continuation of U.S. Patent Application No. 14/155,344, filed January 14, 2014 which claims priority to U.S. Provisional Patent Application Nos. 61/752,348 filed on January 14, 2013, No. 61/780,310 filed on March 13, 2013, No. 61/778,148 filed March 12, 2013, and No. 61/764,954 filed February 14, 2013, the entire contents of which are incorporated herein for all purposes by this reference.

SEQUENCE LISTING

[0002] 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 June 26, 2017, is named 067461-5161-US06_ST25.txt and is 763,759 bytes in size.

INCORPORATION OF RELATED APPLICATIONS

[0003] The following applications are incorporated by reference in their entirety, USSNs 61/302,707, 61/732,813, 61/598,686, 61/441,552, 61/311,472, 61/450,457, 61/545,498, 61/368,969, 61/391,509, 61/391,515, 61/439,263, 61/368,962 and 61/593,846 , 61/515,745, 61/752,349, 13/194,904, 13/568,028, 13/648,951, 12/875,015, as well as US Publication Nos. 2009/0163,699 and 2006/0024298;

TECHNICAL FIELD

[0004] The present invention describes novel immunoglobulin compositions that simultaneously co-engage antigens, where both of the antigens are bound monovalently. The novel immunoglobulins described preferably utilize heterodimeric Fc regions. Methods of using the novel immunoglobulin compositions, particularly for therapeutic purposes, are also described herein.

BACKGROUND OF THE INVENTION

[0005] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer and autoimmune/inflammatory disorders. Yet improvements to this class of drugs are still needed, particularly with respect to enhancing their clinical

efficacy. One avenue being explored is the engineering of additional and novel antigen binding sites into antibody-based drugs such that a single immunoglobulin molecule co-engages two different antigens. Such non-native or alternate antibody formats that engage two different antigens are often referred to as bispecifics. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific generation is the introduction of new variable regions into the antibody.

[0006] A number of alternate antibody formats have been explored for bispecific targeting (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). Initially, bispecific antibodies were made by fusing two cell lines that each produced a single monoclonal antibody (Milstein et al., 1983, Nature 305:537-540). Although the resulting hybrid hybridoma or quadroma did produce bispecific antibodies, they were only a minor population, and extensive purification was required to isolate the desired antibody. An engineering solution to this was the use of antibody fragments to make bispecifics. Because such fragments lack the complex quaternary structure of a full length antibody, variable light and heavy chains can be linked in single genetic constructs. Antibody fragments of many different forms have been generated, including diabodies, single chain diabodies, tandem scFv's, and Fab_2 bispecifics (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). While these formats can be expressed at high levels in bacteria and may have favorable penetration benefits due to their small size, they clear rapidly in vivo and can present manufacturing obstacles related to their production and stability. A principal cause of these drawbacks is that antibody fragments typically lack the constant region of the antibody with its associated functional properties, including larger size, high stability, and binding to various Fc receptors and ligands that maintain long half-life in serum (i.e., the neonatal Fc receptor FcRn) or serve as binding sites for purification (i.e., protein A and protein G).

[0007] More recent work has attempted to address the shortcomings of fragment-based bispecifics by engineering dual binding into full length antibody -like formats (Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297; USSN12/477,711; Michaelson et al., 2009, mAbs 1[2]:128-141; PCT/US2008/074693; Zuo et al., 2000, Protein Engineering 13[5]:361-367; USSN09/865,198; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu et al., 2005,

J Biol Chem 280[20]:19665-19672; PCT/US2005/025472; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). These formats overcome some of the obstacles of the antibody fragment bispecifics, principally because they contain an Fc region. One significant drawback of these formats is that, because they build new antigen binding sites on top of the homodimeric constant chains, binding to the new antigen is always bivalent.

[0008] For many antigens that are attractive as co-targets in a therapeutic bispecific format, the desired binding is monovalent rather than bivalent. For many immune receptors, cellular activation is accomplished by cross-linking of a monovalent binding interaction. The mechanism of cross-linking is typically mediated by antibody/antigen immune complexes, or via effector cell to target cell engagement. For example, the low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, upon immune complexation or cell-to-cell contact, receptors are cross-linked and clustered on the cell surface, leading to activation. For receptors responsible for mediating cellular killing, for example FcγRIIIa on natural killer (NK) cells, receptor cross-linking and cellular activation occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, expressly incorporated by reference). Similarly, on B cells the inhibitory receptor FcγRIIb downregulates B cell activation only when it engages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). As another example, CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Indeed nonspecific bivalent cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622–632; expressly incorporated by reference). Thus for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged target.

[0009] Thus while bispecifics generated from antibody fragments suffer biophysical and pharmacokinetic hurdles, a drawback of those built with full length antibody -like formats is that they engage co-target antigens multivalently in the absence of the primary target antigen, leading to nonspecific activation and potentially toxicity. The present invention solves this problem by introducing a novel bispecific format that enables the co-engagement of distinct target antigens.

SUMMARY OF THE INVENTION

[0010] Accordingly, in one aspect, the invention provides heterodimeric antibodies comprising a first heavy chain comprising a first Fc domain and a single chain Fv region that binds a first antigen. The heterodimeric antibodies also comprise a second heavy chain comprising a second Fc domain, a first variable heavy chain and a first variable light chain, wherein the first and second Fc domains are different.

[0011] In a further aspect, the pIs of the first heavy chain and the second heavy chain are at least 0.5 logs apart.

[0012] In an additional aspect, the heterodimeric antibodies of the invention further comprise one or two additional antigen binding domains that can be either or both of an scFv and a Fab.

[0013] In a further aspect, the heterodimeric antibodies of the invention comprise heterodimerization variants according to Figures 4, 5, 6, 20, 21 and 22.

[0014] In an additional aspect, the heterodimeric antibodies comprise pI variants in each monomer, with monomer one are ISO(-): I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL44 and the pI subsitutions in monomer 2 are ISO(+RR): Q196K/I199T/P217R/P228R/N276K.

[0015] In additional aspects, the present invention provides heterodimeric antibodies wherein one of the heavy chains comprises a variant selected from the group consisting of S119E, K133E, K133Q, R133E (in case of IgG2-4), R133Q (in case of IgG2-4), T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, Deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E, 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S,

370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, 439D, 349C/364E, 349K/351K, 349K/351K/394F, 349K/354C, 349K/394F, 349K/394F/401K, 349K/394Y, 349K/401K, 349K/405A, 349T/351E/411E, 349T/394F, 349T/394F/401K, 349T/394F/411E, 349T/405A, 349T/411E, 351E/364D, 351E/364D/405A, 351E/364E, 351E/366D, 351K/364H/401K, 351K/366K, 364D/370G, 364D/394F, 364E/405A, 364E/405S, 364E/411E, 364E/411E/405A, 364H/394F, 364H/401K, 364H/401K/405A, 364H/405A, 364H/405A/411E, 364Y/370R, 370E/411E, 370R/411K, 395T/397S/405A and 397S/405A.

[0016] The present invention describes methods for generating the novel compositions of the invention. The present invention describes purification methods for the immunogloublins herein, particularly methods for separating heterodimeric and homodimeric protein species. Also described are methods of testing the immunoglobulins herein, including in vitro and in vitro experiments.

[0017] The present invention provides isolated nucleic acids encoding the novel immunoglobulin compositions 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 immunoglobulin compositions.

[0018] The present invention provides compositions comprising immunoglobulin polypeptides described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[0019] The present invention contemplates therapeutic and diagnostic uses for the immunoglobulin polypeptides disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures for bottle cap

[0020] Figure 1A-1C. Illustration of the "triple F" format for bispecific immunoglobulins. Figure 1A shows a scFv-Fc format. Figure 1C depicts a more standard bispecific format, also utilizing the pI variants of the invention (and optionally and independently the other heterodimerization variants). Figure 1B shows the "triple F" format (sometimes also referred to as the "bottle-opener" configuration; (and optionally and independently the other heterodimerization variants).

[0021] Figures 2A-2E depict a variety of "higher multispecificity" embodiments of the present invention. Figure 2A shows a "triple F" configuration with another scFv attached to the Fab monomer (this one, along with Figure 2D, has a greater molecular weight differential as well). Figure 2B depicts a "triple F" with another scFv attached to the scFv monomer. Figure 2C depicts a "three scFv" format. Figure 2D depicts an additional Fab attached to the Fab monomer. Figure 2E depicts a Fab hooked to one of the scFv monomers.

[0022] Figures 3A-3E show additional varieties of "higher multispecificity" embodiments of the "triple F" format, all with one monomer comprising an scFv and all of which have molecular weight differentials which can be exploited for purification of the heterodimers). Figure 3A shows a "Fab-Fv" format with binding to two different antigens, with Figure 3B depicting the "Fab-Fv" format with binding to a single antigen (e.g. bivalent binding to antigen 1). Figures 3C and 3D depicts a "Fv-Fab" format with similar bivalent or monovalent additional antigen binding. Figure 3E depicts one monomer with a CH1-CL attached to the second scFv.

[0023] Figure 4A and 4B show novel steric variants. As will be understood by those in the art, the first column of each table represents "corresponding" monomer pairs or "sets": that is, monomer 1 has 405A and the corresponding steric variant is 394F. Again, these can be optionally and independently combined, as long as some "strandedness" is maintained.

[0024] Figure 5A-5B depicts heterodimerization variants that find particular use in the present invention.

[0025] Figure 6 depicts novel pI heterodimerization variants of particular use in the creation of heterodimeric proteins. As will be understood by those in the art, the first row of each table represents "corresponding" monomer pairs or sets. Note that the I199T and V397M variants are "neutral" (e.g. not for the purposes of altering pI of one of the monomers). Rather, they contribute to the reduction of immunogenicity by helping preserve a close epitope; e..g I199T follows pI variants N203D or Q196K, and V397M follows K392N. Thus these two variants can optionally and independently be removed from any of the sequences above.

[0026] Figure 7A-7B. Amino acid sequences for an anti-CD19 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0027] Figure 8. Amino acid sequences for the humanized anti-CD3 variable regions finding particular use in the invention (with CDRs underlined).

[0028] Figure 9. Production of XENP11874, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 9A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 9B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 9C).

[0029] Figure 10. XENP11874, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for B cell depletion.

[0030] Figure 11. The production of XENP11924, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 11A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 11B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 11C).

[0031] Figure 12. XENP11924, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the Raji tumor cell line.

[0032] Figure 13. Amino acid sequences for the anti-CD38 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0033] Figure 14. The production of XENP11925, a "triple F" bispecific with an anti-CD38 Fab and anti-CD3 scFv. In Fig. 14A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 14B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 14C).

[0034] Figure 15. XENP11925, anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the RPMI8226 tumor cell line.

[0035] Figure 16A-16B. Amino acid sequences for embodiments of the anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain for each bispecific. Amino acid substitutions made to "skew" the

formation toward desired "triple F" heterodimer over undesired dual scFv-Fc and mAb homodimers are underlined.

[0036] Figure 17. Isoelectric focusing (IEF) gel providing analysis of various "triple F" bispecifics. The desired "triple F" heterodimer has an apparent pI of 8.4 (middle band). Note the increase in heterodimer yield for XENP12152 and XENP12155.

[0037] Figure 18. Analytical ion exchange chromatography providing analysis of various "triple F" bispecifics. Yield of the desired "triple F" heterodimer is boxed. Note the increase in heterodimer yield for XENP12155.

[0038] Figure 19 shows the literature pIs of the 20 amino acids. It should be noted that the listed pIs are calculated as free amino acids; the actual pI of any side chain in the context of a protein is different, and thus this list is used to show pI trends and not absolute numbers for the purposes of the invention.

[0039] Figure 20A-20F . Data table of exemplary pI-engineered variants listing:

XenP#	the internal reference number
Name (HC)	heavy chain sequence designation
SEQ ID NO (HC)	corresponding SEQ ID NO of the heavy chain sequence
Name (LC)	light chain sequence designation
SEQ ID NO (LC)	corresponding SEQ ID NO of the light chain sequence
Calc. pI	calculated pI value for the entire antibody sequence, including heavy and light chain Fv + constant domains, with the Fv of bevacizumab and the constant domains as defined in the table
#KR	number of Lys or Arg residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta KR (vs. WT)	change in the number of Lys or Arg residues relative to IgG1 wild-type sequence of bevacizumab
#DE	number of Asp or Glu residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta DE (vs. WT)	change in the number of Asp or Glu acid residues relative to IgG1 wild-type sequence of bevacizumab
Charge state	derived from the total number of Lys and Arg minus the total number of Asp and Glu residues, assuming a pH of 7
# HC Mutations vs IgG1	number of mutations in the heavy chain constant domain as compared to IgG1
# LC Mutations vs IgG1	number of mutations in the light chain constant domain as compared to IgG1
Total # of Mutations	total number of mutations in the heavy chain and light chain constant domains as compared to IgG1

[0040] It should be noted that Figure 20A-20F has SEQ ID NO:s that are associated with the sequence listing filed in USSN 13/648,951, and are hereby expressly incorporated by reference.

[0001] Figure 21A-21C. depicts a number of pI variants that can additionally find use in the present invention, which is a list of all possible reduced pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0041] Figure 22 List of all possible increased pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0042] Figure 23A-23B is an additional list of potential heterodimerization variants for use in the present invention.

[0043] Figure 24 depicts a matrix of possible combinations of heterodimerization formats, heterodimerization variants (separated into pI variants and steric variants (which includes charge pair variants), Fc variants, FcRn variants and combinations. Legend A are suitable FcRn variants: 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E and 259I/308F/428L. That is, the Triple F format of Figure 1B can have any of these FcRn variants. For clarity, as each heavy chain is different, FcRn variants (as well as the Fc variants) can reside on one or both monomers. Legend B are suitable Fc variants: 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T. (Note, additional suitable Fc variants are found in Figure 41 of US 2006/0024298, the figure and legend of which are hereby incorporated by reference in their entirety). Legend C are suitable pI variants, and these, for brevity are imported from Figure 23A-23B, again with the understanding that there is a "strandedness" to pI variants. Legend D are suitable steric variants (including charge pair variants); again, for brevity are imported from Figure 21A-21C, again with the understanding that there is a "strandedness" to steric variants. Legend E reflects the following possible combinations, again, with each variant being independently and optionally combined from

the appropriate source Legend: 1) pI variants plus FcRn variants; 2) pI variants plus Fc variants; 3) pI variants plus FcRn variants plus Fc variants; 4) steric variants plus FcRn variants; 5) steric variants plus Fc variants; 6) steric variants plus FcRn variants plus Fc variants; 7) pI variants plus steric variants plus FcRn variants; 8) pI variants plus steric variants plus Fc variants; 9) pI variants plus steric variants plus FcRn variants plus Fc variants; and 10) pI variants plus steric variants.

[0044] Figures 25A, 25B and 25C depict stability-optimized, humanized anti-CD3 variant scFvs. Substitutions are given relative to the H1_L1.4 scFv sequence. Amino acid numbering is Kabat numbering.

[0045] Figures 26A to 26YY depict the amino acid sequences of stability-optimized, humanized anti-CD3 variant scFvs, variable heavy and variable light sequences. (Note also that the first sequence is the histidine tagged version for ease of purification). CDRs are underlined. It should be understood that the increased stability of the optimized variable and optimized light chains (as well as the scFv chains) can be attributed to framework regions as well as the CDRs. Thus, it should be understood that the disclosure of the entire variable region includes the disclosure of the framework regions, although they are not separately numbered. In addition, the scFv linkers are shown in grey. Each scFv linker can be replaced with a charged scFv linker as depicted in Figure 7A-7B. That is, any charged scFv linker, whether positive or negative, including those depicted in Figure 7A-7B, can be substituted for the highlighted region in Figures 2A to 2YY.

[0046] Figures 27A and 27B show yields after Ni-NTA purification and melting temperatures (Tm) as determined by DSF (Differential Scanning Fluorimetry) of stability-optimized, humanized anti-CD3 variant scFvs. Similar DSF stability measurements for the I2C anti-CD3 variable region in a comparable scFv format gave a Tm of 59°C.

[0047] Figures 28A and 28B show fold improvement (relative to H1_L1.4 scFv) in cell surface binding affinities (IC50) of stability-optimized, humanized anti-CD3 variant scFvs as determined in a competitive binding experiment using purified human T cells from PBMCs.

[0048] Figure 29. Figure depicts the sequence of human CD3.

[0049] Figures 30A to 30I depict a collation of all the CD3 vhCDR1-3 and vlCDR1-3 sequences useful in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0050] Figures 1-76 of USSN 61/593846 and the associated legends and discussion in the specification are hereby incorporated by reference.

[0051] Figure 2 and 17 of USSN 61/778,157, inclusive of all the sequences including the optimized CD3 sequences are expressly incorporated herein by reference.

I. Overview

[0052] The present invention is directed to novel constructs to provide bispecific antibodies (or, as discussed below, trispecific or tetraspecific antibodies can also be made). An ongoing problem in antibody technologies is the desire for "bispecific" (and/or multispecific) antibodies that bind to two (or more) different antigens simultaneously, in general thus allowing the different antigens to be brought into proximity and resulting in new functionalities and new therapies. In general, these antibodies are made by including genes for each heavy and light chain into the host cells. This generally results in the formation of the desired heterodimer (A-B), as well as the two homodimers (A-A and B-B). However, a major obstacle in the formation of multispecific antibodies is the difficulty in purifying the heterodimeric antibodies away from the homodimeric antibodies and/or biasing the formation of the heterodimer over the formation of the homodimers.

[0053] The present invention is generally directed to the creation of heterodimeric proteins such as antibodies that can co-engage antigens in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers.

[0054] Thus, the present invention is directed to novel immunoglobulin compositions that co-engage at least a first and a second antigen. First and second antigens of the invention are herein referred to as antigen-1 and antigen-2 respectively. One heavy chain of the antibody contains an single chain Fv ("scFv", as defined below) and the other heavy chain is a "regular" FAb format, comprising a variable heavy chain and a light chain. This structure is sometimes referred to herein as "triple F" format (sc**F**v-**F**Ab-**F**c) or the "bottle-opener" format, due to a rough visual similarity to a bottle-opener (see Figure 1A-1C). The two chains are brought together by the use of amino acid variants in the constant regions (e.g. the Fc domain and/or the hinge region) that promote the formation of heterodimeric antibodies as is described more fully below.

[0055] There are several distinct advantages to the present "triple F" format. As is known in the art, antibody analogs relying on two scFv constructs often have stability and aggregation problems, which can be alleviated in the present invention by the addition of a "regular" heavy and light chain pairing. In addition, as opposed to formats that rely on two heavy chains and two light chains, there is no issue with the incorrect pairing of heavy and light chains (e.g. heavy 1 pairing with light 2, etc.)

[0056] There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art and described more fully below, these mechanisms can be combined to ensure high heterodimerization.

[0057] One mechanism is generally referred to in the art as "knobs and holes" ("KIH"), referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figure 4A and 4B, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization. Some of these variants are shown in Figures 16, 20, 21 and 22.

[0055] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0056] In the present invention, there are two basic mechanisms that can lead to ease of purifying heterodimeric proteins; one relies on the use of pI variants, such that each monomer has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, the "triple F" format also allows separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers, as is generally outlined below.

[0057] In the present invention that utilizes pI as a separation mechanism to create the heterodimeric Triple F format, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As is outlined more fully below, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g. a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g. glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g. loss of a charge; lysine to serine). A number of these variants are shown in Figures 6, 20 and 21.

[0058] Accordingly, in this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease it's pI (wt A-+B or wt A - -B), or by increasing one region and decreasing the other region (A+ -B- or A- B+). It should be noted that in this discussion it does not matter which monomer comprises the scFv and which the Fab.

[0059] Thus, in general, a component of the present invention are amino acid variants in the constant regions of antibodies that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein to form "pI heterodimers" (when the protein is an antibody, these are referred to as "pI antibodies") by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[0060] Furthermore, as will be appreciated by those in the art and outlined herein, heterodimers can be separated from homodimers on the basis of size. For example, as shown in Figure 1A-1B, heterodimers with two scFvs (Figure 1A) can be separated by those of the "triple F" format (figure 1B) and a bispecific mAb (Figure 1C). This can be further exploited in higher valency with additional antigen binding sites being utilized. For example, in as shown in Figure 2D, one monomer will have two Fab fragments and the other will have one scFv, resulting in a differential in size and thus molecular weight. Other examples are shown in Figures 2 and 3.

[0061] In addition, as will be appreciated by those in the art and outlined herein, the format outlined herein can be expanded to provide trispecific and tetraspecific antibodies as well. In this embodiment, some variations of which are depicted in Figures 2 and 3, it will be recognized that it is possible that some antigens are bound divalently (e.g. two antigen binding sites to a single antigen; for example, A and B could be part of a typical bivalent association and C and D can be optionally present and optionally the same or different). As will be appreciated, any combination of Fab and scFvs can be utilized to achieve the desired result and combinations.

[0062] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of the heavy chain(s), a more modular approach to designing and purifying multispecific proteins, including antibodies, is provided. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[0063] In one embodiment, the heterodimeric antibody provides for monovalent engagement of one antigen using a scFv and monovalent engagement of the other antigen using a FAb. As outlined below, this format can also be varied; in some embodiments, there is monovalent engagement of three antigens, divalent engagement of one antigen and monovalent engagement of a second antigen (e.g. A and C are to the same antigen and B is to a different antigen), etc.

[0064] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904

(incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[0065] In addition to all or part of a variant heavy constant domain, one or both of the monomers may contain one or two fusion partners, such that the heterodimers form multivalent proteins. As is generally depicted in the Figures, the fusion partners are depicted as A, B, C and D, with all combinations possible. In general, A, B, C and D are selected such that the heterodimer is at least bispecific or bivalent in its ability to interact with additional proteins. In the context of the present "triple F" format, generally A and B are an scFv and a Fv (as will be appreciated, either monomer can contain the scFv and the other the Fv/Fab) and then optionally one or two additional fusion partners.

[0066] Accordingly, the present invention provides novel constructs to produce multivalent antibodies.

[0067] Furthermore, as outlined herein, additional amino acid variants may be introduced into the bispecific antibodies of the invention, to add additional functionalities. For example, amino acid changes within the Fc region can be added (either to one monomer or both) to facilitiate increased ADCC or CDC (e.g. altered binding to Fcγ receptors); to allow or increase yield of the addition of toxins and drugs (e.g. for ADC), as well as to increase binding to FcRn and/or increase serum half-life of the resulting molecules. As is further described herein and as will be appreciated by those in the art, any and all of the variants outlined herein can be optionally and independently combined with other variants.

II. Definitions

[0068] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[0069] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding is the double variant 236R/328R.

[0070] 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. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[0071] 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.

[0072] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[0073] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0074] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position

234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[0075] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233# designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[0076] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides". The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity . Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The

identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[0077] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise 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), entirely incorporated by reference). The amino acids may either be naturally occurring or synthetic (e.g. not an amino acid that is coded for by DNA); as will be appreciated by those in the art. For example, homo-phenylalanine, citrulline, ornithine and noreleucine are considered synthetic amino acids for the purposes of the invention, and both D- and L-(R or S) configured amino acids may be utilized. The variants of the present invention may comprise modifications that include the use of synthetic amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson et al., 2004, Proc Natl Acad Sci USA 101 (2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, Science 301(5635):964-7, all entirely incorporated by reference. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[0078] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[0079] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[0080] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[0081] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[0082] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[0083] 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.

[0084] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. 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/Fc ligand complex.

[0085] By "Fc gamma receptor", "FcγR" or "FcqammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. 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 FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), 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.

[0086] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life, are discussed in Legend A of Figure 24.

[0087] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The 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 immunoglobulin" as used herein is meant an unmodified immunoglobulin 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. It should be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[0088] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as a binding moiety to a target protein, as described herein)..

[0089] 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 for antibody numbering.

[0090] By "strandedness" in the context of the monomers of the heterodimeric proteins of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities.

[0091] 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. A wide number of suitable target antigens are described below.

[0092] By "target cell" as used herein is meant a cell that expresses a target antigen.

[0093] 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.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[0094] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

Heterodimeric Proteins

[0095] The present invention is directed to the generation of multispecific, particularly bispecific binding proteins, and in particular, multispecific antibodies that have one monomer comprising an scFv and the other an Fv.

Antibody

[0096] The present invention relates to the generation of multispecific antibodies, generally therapeutic antibodies. As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. In general, the term "antibody" includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL.

[0097] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the present invention covers pI engineering of IgG1/G2 hybrids.

[0098] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[0099] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00100] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00101] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat et al., supra (1991)).

[00102] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00103] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00104] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a

polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00105] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning."

[00106] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[0100] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[0101] It should be noted that the sequences depicted herein start at the CH1 region, position 118; the variable regions are not included except as noted. For example, the first amino acid of SEQ ID NO: 2, while designated as position"1" in the sequence listing, corresponds to position 118 of the CH1 region, according to EU numbering.

[0102] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the

numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[0103] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[0104] Another region of interest for additional substitutions, outlined below, is the Fc region. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, 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, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0105] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[0106] Alternatively, the antibodies can include a variety of structures, including, but not limited to, antibody fragments,, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[0107] In one embodiment, the antibody is an antibody fragment, as long as it contains at least one constant domain which can be engineered to produce heterodimers, such as pI engineering. Other antibody fragments that can be used include fragments that contain one or

more of the CH1, CH2, CH3, hinge and CL domains of the invention that have been pI engineered. For example, Fc fusions are fusions of the Fc region (CH2 and CH3, optionally with the hinge region) fused to another protein. A number of Fc fusions are known the art and can be improved by the addition of the heterodimerization variants of the invention. In the present case, antibody fusions can be made comprising CH1; CH1, CH2 and CH3; CH2; CH3; CH2 and CH3; CH1 and CH3, any or all of which can be made optionally with the hinge region, utilizing any combination of heterodimerization variants described herein.

[0108] In some embodiments of the present invention, one monomer comprises a heavy chain comprises a scFV linked to an Fc domain, and the other monomer comprises a heavy chain comprising a Fab linked to an Fc domain, e.g. a "typical" heavy chain, and a light chain. By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

Chimeric and Humanized Antibodies

[0109] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely

incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

Multispecific Antibody Constructs

[0110] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention take on a number variety of configurations, with a preferred embodiment shown in Figure 1B as a "triple F" construct.

Heterodimeric Heavy Chain Constant Regions

[0111] Accordingly, the present invention provides heterodimeric proteins based on the use of monomers containing variant heavy chain constant regions as a first domain. By "monomer" herein is meant one half of the heterodimeric protein. It should be noted that traditional antibodies are actually tetrameric (two heavy chains and two light chains). In the context of the present invention, one pair of heavy-light chains (if applicable, e.g. if the monomer comprises an Fab) is considered a "monomer". Similarly, a heavy chain region comprising the scFv is considered a monomer. In the case where an Fv region is one fusion partner (e.g. heavy and light chain) and a non-antibody protein is another fusion partner, each "half" is considered a monomer. Essentially, each monomer comprises sufficient heavy chain constant region to allow heterodimerization engineering, whether that be all the constant region, e.g. Ch1-hinge-CH2-CH3, the Fc region (CH2-CH3), or just the CH3 domain.

[0112] The variant heavy chain constant regions can comprise all or part of the heavy chain constant region, including the full length construct, CH1-hinge-CH2-CH3, or portions thereof, including for example CH2-CH3 or CH3 alone. In addition, the heavy chain region of each monomer can be the same backbone (CH1-hinge-CH2-CH3 or CH2-CH3) or different. N- and C-terminal truncations and additions are also included within the definition; for example, some pI variants include the addition of charged amino acids to the C-terminus of the heavy chain domain.

[0113] Thus, in general, one monomer of the present "triple F" construct is a scFv region-hinge-Fc domain) and the other is (VH-CH1-hinge- CH2-CH3 plus associated light chain), with heterodimerization variants, including steric and pI variants, Fc and FcRn variants, and additional antigen binding domains (with optional linkers) included in these regions.

[0114] In addition to the heterodimerization variants (e.g. steric and pI variants) outlined herein, the heavy chain regions may also contain additional amino acid substitutions, including changes for altering FcγR and FcRn binding as discussed below.

[0115] In addition, some monomers can utilize linkers between the variant heavy chain constant region and the fusion partner. For the scFv portion of the "bottle-opener", standard linkers as are known in the art can be used. In the case where additional fusion partners are made (e.g. Figures 2and 3), traditional peptide linkers can be used, including flexible linkers of glycine and serine. In some cases, the linkers for use as components of the monomer are

different from those defined below for the ADC constructs, and are in many embodiments not cleavable linkers (such as those susceptible to proteases), although cleavable linkers may find use in some embodiments.

[0116] The heterodimerization variants include a number of different types of variants, including, but not limited to, steric variants (including charge variants) and pI variants, that can be optionally and independently combined with any other variants. In these embodiments, it is important to match "monomer A" with "monomer B"; that is, if a heterodimeric protein relies on both steric variants and pI variants, these need to be correctly matched to each monomer: e.g. the set of steric variants that work (1 set on monomer A, 1 set on monomer B) is combined with pI variant sets (1 set on monomer A, 1 set on monomer B), such that the variants on each monomer are designed to achieve the desired function.

[0117] It is important to note that the heterodimerization variants outlined herein (for example, including but not limited to those variants shown in Figures 4, 5 and 6), can be optionally and independently combined with any other variants, and on any other monomer. Thus, for example, pI variants for monomer 1 from Figure 6 can be added to other heterodimerization variants for monomer 1 in Figures 5A-5B and 6 or from monomer 2. That is, what is important for the heterodimerization is that there are "sets" of variants, one set for one monomer and one set for the other. Whether these are combined from the Figures 1 to 1 (e.g. monomer 1 listings can go together) or switched (monomer 1 pI variants with monomer 2 steric variants) is irrelevant. However, as noted herein, "strandedness" should be preserved when combinations are made as outlined above. Furthermore, for the additional Fc variants (such as for FcγR binding, FcRn binding, etc.), either monomer, or both monomers, can include any of the listed variants, independently and optionally. In some cases, both monomers have the additional variants and in some only one monomer has the additional variants, or they can be combined.

Heterodimerization Variants

[0118] The present invention provides multispecific antibody formats, on a "triple F" or "bottle opener" scaffold as depicted in Figure 1~~B and Figures 2 and 3~~A-3E.

Steric Variants

[0119] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the heterodimeric structure than to form

homodimers with the same Fc amino acid sequences. Suitable steric variants are shown in Figures 4 and 5.

[0120] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figures 4 and 5, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[0121] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0122] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876, the figure and legend of which are incorporated expressly by reference herein.

[0123] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers.

pI (Isoelectric point) Variants for Heterodimers

[0124] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[0125] Preferred combinations of pI variants are shown in Figure 6, with additional pI variants disclosed in Figures 20, 21 and 22.

Heavy Chain Acidic pI Changes

[0126] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more positive (e.g. lower the pI), one or more of the following substitutions can be made: S119E, K133E, K133Q, T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, a deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

[0127] In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

Basic pI changes

[0128] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more negative (e.g. increase the pI), one or more of the following substitutions can be made: Q196K, P217R, P228R, N276K and H435R. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

Antibody Heterodimers Light chain variants

[0129] In the case of antibody based heterodimers, e.g. where at least one of the monomers comprises a light chain in addition to the heavy chain domain, pI variants can also be made in the light chain. Amino acid substitutions for lowering the pI of the light chain include, but are not limited to, K126E, K126Q, K145E, K145Q, N152D, S156E, K169E, S202E, K207E and adding peptide DEDE at the c-terminus of the light chain. Changes in this category based on the constant lambda light chain include one or more substitutions at R108Q, Q124E,

K126Q, N138D, K145T and Q199E. In addition, increasing the pI of the light chains can also be done.

Isotypic Variants

[0130] In addition, many embodiments of the invention rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significant affect the pI of the variant antibody. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[0131] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g. by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[0132] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

Calculating pI

[0133] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figures. Alternatively, the pI of each monomer can be compared.

pI Variants that also confer better FcRn in vivo binding

[0134] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[0135] Although still under examination, Fc regions are believed to have longer half-lives *in vivo*, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice, Dall' Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half life as wild-type Fc (Dall' Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life *in vivo* will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[0136] Recently it has been suggested that antibodies with variable regions that have lower isoelectric points may also have longer serum half-lives (Igawa et al., 2010 PEDS. 23(5): 385-392, entirely incorporated by reference). However, the mechanism of this is still poorly understood. Moreover, variable regions differ from antibody to antibody. Constant region variants with reduced pI and extended half-life would provide a more modular approach to improving the pharmacokinetic properties of antibodies, as described herein.

[0137] pI variants that find use in this embodiment, as well as their use for purification optimization, are disclosed in Figure 20A-20F.

Combination of Heterodimeric Variants

[0138] As will be appreciated by those in the art, all of the recited heterodimerization variants can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the hterodimerization formats. See Figure 24.

[0139] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

Target Antigens

[0140] The immunoglobulins of the invention may target virtually any antigens. The "triple F" format is particularly beneficial for targeting two (or more) distinct antigens. (As outlined

herein, this targeting can be any combination of monovalent and divalent binding, depending on the format). Thus the immunoglobulins herein preferably co-engage two target antigens, although in some cases, three or four antigens can be monovalently engaged. Each monomer's specificity can be selected from the lists below. While the triple F immunoglobulins described herein are particularly beneficial for targeting distinct antigens, in some cases it may be beneficial to target only one antigen. That is, each monomer may have specificity for the same antigen.

[0141] Particular suitable applications of the immunoglobulins herein are co-target pairs for which it is beneficial or critical to engage each target antigen monovalently. Such antigens may be, for example, immune receptors that are activated upon immune complexation. Cellular activation of many immune receptors occurs only by cross-linking, acheived typically by antibody/antigen immune complexes, or via effector cell to target cell engagement. For some immune receptors, for example the CD3 signaling receptor on T cells, activation only upon engagement with co-engaged target is critical, as nonspecifiic cross-linking in a clinical setting can elicit a cytokine storm and toxicity. Therapeutically, by engaging such antigens monovalently rather than multivalently, using the immunoglobulins herein, such activation occurs only in response to cross-linking only in the microenvironment of the primary target antigen. The ability to target two different antigens with different valencies is a novel and useful aspect of the present invention. Examples of target antigens for which it may be therapeutically beneficial or necessary to co-engage monovalently include but are not limited to immune activating receptors such as CD3, FcγRs, toll-like receptors (TLRs) such as TLR4 and TLR9, cytokine, chemokine, cytokine receptors, and chemokine receptors. In many embodiments, one of the antigen binding sites binds to CD3, and in some embodiments it is the scFv-containing monomer.

[0142] Virtually any antigen may be targeted by the immunoglobulins herein, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ,

APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3),

GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PlGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN,

R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIb, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1,

VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0143] Exemplary antigens that may be targeted specifically by the immunoglobulins of the invention include but are not limited to: CD20, CD19, Her2, EGFR, EpCAM, CD3, FcγRIIIa (CD16), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRI (CD64), Toll-like receptors (TLRs) such as TLR4 and TLR9, cytokines such as IL-2, IL-5, IL-13, IL-12, IL-23, and TNFα, cytokine receptors such as IL-2R, chemokines, chemokine receptors, growth factors such as VEGF and HGF, and the like. . To form the multispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0144] Particularly preferred combinations for bispecific antibodies are an antigen-binding domain to CD3 and an antigen binding domain to CD19; an antigen-binding domain to CD3 and an antigen binding domain to CD33; an antigen-binding domain to CD3 and an antigen binding domain to CD 38. Again, in many embodiments, the CD3 binding domain is the scFv, having an exemplary sequence as depicted in the Figures and/or CD3 CDRs as outlined.

[0145] The choice of suitable target antigens and co-targets depends on the desired therapeutic application. Some targets that have proven especially amenable to antibody therapy are those with signaling functions. Other therapeutic antibodies exert their effects by blocking signaling of the receptor by inhibiting the binding between a receptor and its cognate ligand. Another mechanism of action of therapeutic antibodies is to cause receptor down regulation. Other antibodies do not work by signaling through their target antigen. The choice of co-targets will depend on the detailed biology underlying the pathology of the indication that is being treated.

[0146] Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (Weiner et al., 2010, Nature Reviews Immunology 10:317-327; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). For anti-cancer treatment it may be desirable to target one antigen (antigen-1) whose expression is restricted to the cancerous cells while co-targeting a second antigen (antigen-2) that mediates some immunulogical killing activity. For other treatments it may be beneficial to co-target two antigens, for example two angiogenic factors or two growth factors, that are each known to play some role in proliferation of the tumor. Exemplary co-targets for oncology include but are not limited to HGF and VEGF, IGF-1R and VEGF, Her2 and VEGF, CD19 and CD3, CD20 and CD3, Her2 and CD3, CD19 and FcγRIIIa, CD20 and FcγRIIIa, Her2 and FcγRIIIa. An immunoglobulin of the invention may be capable of binding VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and CD4; HGF and c-MET; HGF and NRP1; HGF and phosphatidylserine; ErbB3 and IGF1R; ErbB3 and IGF1,2; c-Met and Her-2; c-Met and NRP1; c-Met and IGF1R; IGF1,2 and PDGFR; IGF1,2 and CD20; IGF1,2 and IGF1R; IGF2 and EGFR; IGF2 and HER2; IGF2 and CD20; IGF2 and VEGF; IGF2 and IGF1R; IGF1 and IGF2; PDGFRa and VEGFR2; PDGFRa and PLGF; PDGFRa and VEGF; PDGFRa and c-Met; PDGFRa and EGFR; PDGFRb and VEGFR2; PDGFRb and c-Met; PDGFRb and EGFR; RON and c-Met; RON and MTSP1; RON and MSP; RON and CDCP1; VGFR1 and PLGF; VGFR1 and RON; VGFR1 and EGFR; VEGFR2 and PLGF; VEGFR2 and NRP1; VEGFR2 and RON; VEGFR2 and DLL4; VEGFR2 and EGFR; VEGFR2 and ROBO4; VEGFR2 and CD55; LPA and S1P; EPHB2 and RON; CTLA4 and VEGF; CD3 and EPCAM; CD40 and IL6; CD40 and IGF; CD40 and CD56; CD40 and CD70; CD40 and VEGFR1; CD40 and DR5; CD40 and DR4; CD40 and APRIL; CD40 and BCMA; CD40 and RANKL; CD28 and MAPG; CD80 and CD40; CD80 and CD30; CD80 and CD33; CD80 and CD74; CD80 and CD2; CD80 and CD3; CD80 and CD19; CD80 and CD4; CD80 and CD52; CD80 and VEGF; CD80 and DR5; CD80 and VEGFR2; CD22 and CD20; CD22 and CD80; CD22 and CD40; CD22 and CD23; CD22 and CD33; CD22 and CD74; CD22 and CD19; CD22 and DR5; CD22 and DR4; CD22 and VEGF; CD22 and CD52; CD30 and CD20;

CD30 and CD22; CD30 and CD23; CD30 and CD40; CD30 and VEGF; CD30 and CD74; CD30 and CD19; CD30 and DR5; CD30 and DR4; CD30 and VEGFR2; CD30 and CD52; CD30 and CD4; CD138 and RANKL; CD33 and FTL3; CD33 and VEGF; CD33 and VEGFR2; CD33 and CD44; CD33 and DR4; CD33 and DR5; DR4 and CD137; DR4 and IGF1,2; DR4 and IGF1R; DR4 and DR5; DR5 and CD40; DR5 and CD137; DR5 and CD20; DR5 and EGFR; DR5 and IGF1,2; DR5 and IGFR, DR5 and HER-2, and EGFR and DLL4. Other target combinations include one or more members of the EGF/erb-2/erb-3 family.

[0147] Other targets (one or more) involved in oncological diseases that the immunoglobulins herein may bind include, but are not limited to those selected from the group consisting of: CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, 1L2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1R, IL2, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3, NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2, NR1D2, NR1H2, NR1H4, NR112, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6 μl, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAU, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB21P, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1,

GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33 μl, SLC43 μl, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB 1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB 1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1, CCL2, CDH5, COL1A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p161NK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (GF), NGFR, NME1 (M23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1), TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase Iia), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1, ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94), RON, c-Met, CD64, DLL4, PLGF, CTLA4, phophatidylserine, ROBO4, CD80, CD22, CD40, CD23, CD28, CD80, CD55, CD38, CD70, CD74, CD30, CD138, CD56, CD33, CD2, CD137, DR4, DR5, RANKL, VEGFR2, PDGFR, VEGFR1, MTSP1, MSP, EPHB2, EPHA1, EPHA2, EpCAM, PGE2, NKG2D, LPA, SIP, APRIL, BCMA, MAPG, FLT3, PDGFR alpha, PDGFR beta, ROR1, PSMA, PSCA, SCD1, and CD59. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0148] Monoclonal antibody therapy has become an important therapeutic modality for treating autoimmune and inflammatory disorders (Chan & Carter, 2010, Nature Reviews

Immunology 10:301-316; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). Many proteins have been implicated in general autoimmune and inflammatory responses, and thus may be targeted by the immunogloublins of the invention. Autoimmune and inflammatory targets include but are not limited to C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (1-TAC/IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78/LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8, CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1), IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5, IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R, ABCF1, BCL6, C3, C4A, CEBPB, CRP, ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP, FADD, IRAK1, IRAK2, MYD88, NCK2, TNFAIP3, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, CD28, CD3E, CD3G, CD3Z, CD69, CD80, CD86, CNR1, CTLA4, CYSLTR1, FCER1A, FCER2, FCGR3A, GPR44, HAVCR2, OPRD1, P2RX7, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, BLR1, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CL1, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCL13, CXCR4, GPR2, SCYE1, SDF2, XCL1, XCL2, XCR1, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, C19orf10 (IL27w), CER1, CSF1, CSF2, CSF3, DKFZp451J0118, FGF2, GFI1, IFNA1, IFNB1, IFNG, IGF1, IL1A, IL1B, IL1R1, IL1R2, IL2, IL2RA, IL2RB, IL2RG, IL3, IL4, IL4R, IL5, IL5RA, IL6, IL6R, IL6ST, IL7, IL8, IL8RA, IL8RB, IL9, IL9R, IL10, IL10RA, IL10RB, IL11, IL12RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17, IL17R, IL18, IL18R1, IL19, IL20, KITLG, LEP, LTA, LTB, LTB4R, LTB4R2, LTBR, MIF, NPPB, PDGFB, TBX21, TDGF1, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFB1, TGFBR1, TGFBR2, TGFBR3, TH1L, TNF, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF11A, TNFRSF21, TNFSF4, TNFSF5, TNFSF6, TNFSF11, VEGF, ZFPM2, and RNF110 (ZNF144). To form the bispecific or trispecific antibodies of the

invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0149] Exemplary co-targets for autoimmune and inflammatory disorders include but are not limited to IL-1 and TNFalpha, IL-6 and TNFalpha, IL-6 and IL-1, IgE and IL-13, IL-1 and IL-13, IL-4 and IL-13, IL-5 and IL-13, IL-9 and IL-13, CD19 and FcγRIIb, and CD79 and FcγRIIb.

[0150] Immunglobulins of the invention with specificity for the following pairs of targets to treat inflammatory disease are contemplated: TNF and IL-17A; TNF and RANKL; TNF and VEGF; TNF and SOST; TNF and DKK; TNF and alphaVbeta3; TNF and NGF; TNF and IL-23p19; TNF and IL-6; TNF and SOST; TNF and IL-6R; TNF and CD-20; IgE and IL-13; IL-13 and IL23p19; IgE and IL-4; IgE and IL-9; IgE and IL-9; IgE and IL-13; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-9; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-23p19; IL-13 and IL-9; IL-6R and VEGF; IL-6R and IL-17A; IL-6R and RANKL; IL-17A and IL-1beta; IL-1beta and RANKL; IL-1beta and VEGF; RANKL and CD-20; IL-1alpha and IL-1beta; IL-1alpha and IL-1beta.

[0151] Pairs of targets that the immunoglobulins described herein can bind and be useful to treat asthma may be determined. In an embodiment, such targets include, but are not limited to, IL-13 and IL-1beta, since IL-1beta is also implicated in inflammatory response in asthma; IL-13 and cytokines and chemokines that are involved in inflammation, such as IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-25; IL-13 and TARC; IL-13 and MDC; IL-13 and MIF; IL-13 and TGF-β; IL-13 and LHR agonist; IL-13 and CL25; IL-13 and SPRR2a; IL-13 and SPRR2b; and IL-13 and ADAM8. The immunoglobulins herein may have specifity for one or more targets involved in asthma selected from the group consisting of CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FGF2, IFNA1, IFNB1, IFNG, histamine and histamine receptors, IL1A, IL1B, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12A, IL12B, IL13, IL14, IL15, IL16, IL17, IL18, IL19, KITLG, PDGFB, IL2RA, IL4R, IL5RA, IL8RA, IL8RB, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL18R1, TSLP, CCLi, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, CCL19, CCL20, CCL22, CCL24,CX3CL1, CXCL1, CXCL2, CXCL3, XCLi, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CX3CR1, GPR2, XCR1, FOS, GATA3, JAK1, JAK3, STAT6, TBX21, TGFB1, TNF, TNFSF6, YY1, CYSLTR1, FCER1A, FCER2, LTB4R, TB4R2, LTBR, and Chitinase. To form the bispecific or trispecific antibodies of the invention, antibodies to any

combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0152] Pairs of targets involved in rheumatoid arthritis (RA) may be co-targeted by the invention, including but not limited to TNF and IL-18; TNF and IL-12; TNF and IL-23; TNF and IL-1beta; TNF and MIF; TNF and IL-17; and TNF and IL-15.

[0153] Antigens that may be targeted in order to treat systemic lupus erythematosus (SLE) by the immunoglobulins herein include but are not limited to CD-20, CD-22, CD-19, CD28, CD4, CD80, HLA-DRA, IL10, IL2, IL4, TNFRSF5, TNFRSF6, TNFSF5, TNFSF6, BLR1, HDAC4, HDAC5, HDAC7A, HDAC9, ICOSL, IGBP1, MS4A1, RGSI, SLA2, CD81, IFNB1, IL10, TNFRSF5, TNFRSF7, TNFSF5, AICDA, BLNK, GALNAC4S-6ST, HDAC4, HDAC5, HDAC7A, HDAC9, IL10, IL11, IL4, INHA, INHBA, KLF6, TNFRSF7, CD28, CD38, CD69, CD80, CD83, CD86, DPP4, FCER2, IL2RA, TNFRSF8, TNFSF7, CD24, CD37, CD40, CD72, CD74, CD79A, CD79B, CR2, ILIR2, ITGA2, ITGA3, MS4A1, ST6GALI, CDIC, CHSTIO, HLA-A, HLA-DRA, and NT5E.; CTLA4, B7.1, B7.2, BlyS, BAFF, C5, IL-4, IL-6, IL-10, IFN-α, and TNF-α. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0154] The immunoglobulins herein may target antigens for the treatment of multiple sclerosis (MS), inlcuding but not limited to IL-12, TWEAK, IL-23, CXCL13, CD40, CD40L, IL-18, VEGF, VLA-4, TNF, CD45RB, CD200, IFNgamma, GM-CSF, FGF, C5, CD52, and CCR2. An embodiment includes co-engagement of anti-IL-12 and TWEAK for the treatment of MS.

[0155] One aspect of the invention pertains to immunoglobulins capable of binding one or more targets involved in sepsis, in an embodiment two targets, selected from the group consisting TNF, IL-1, MIF, IL-6, IL-8, IL-18, IL-12, IL-23, FasL, LPS, Toll-like receptors, TLR-4, tissue factor, MIP-2, ADORA2A, CASP1, CASP4, IL-10, IL-1B, NFκB1, PROC, TNFRSFIA, CSF3, CCR3, ILIRN, MIF, NFκB1, PTAFR, TLR2, TLR4, GPR44, HMOX1, midkine, IRAK1, NFκB2, SERPINA1, SERPINE1, and TREM1. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be

made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0156] In some cases, immunoglobulins herein may be directed against antigens for the treatment of infectious diseases.

[0157] The antibodies of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities.

[0158] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[0159] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10-4 M, at least about 10-5 M, at least about 10-6 M, at least about 10-7 M, at least about 10-8 M, at least about 10-9 M, alternatively at least about 10-10 M, at least about 10-11 M, at least about 10-12 M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[0160] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.
Modified Antibodies

[0161] In addition to the modifications outlined above, other modifications can be made. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996, Nature Biotech. 14:1239-1245, entirely

incorporated by reference). In addition, there are a variety of covalent modifications of antibodies that can be made as outlined below.

[0162] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[0163] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues may also be derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole and the like.

[0164] In addition, modifications at cysteines are particularly useful in antibody-drug conjugate (ADC) applications, further described below. In some embodiments, the constant region of the antibodies can be engineered to contain one or more cysteines that are particularly "thiol reactive", so as to allow more specific and controlled placement of the drug moiety. See for example US Patent No. 7,521,541, incorporated by reference in its entirety herein.

[0165] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

[0166] Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[0167] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin.

Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[0168] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using 125I or 131I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[0169] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N--R'), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[0170] Derivatization with bifunctional agents is useful for crosslinking antibodies to a water-insoluble support matrix or surface for use in a variety of methods, in addition to methods described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cynomolgusogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440, all entirely incorporated by reference, are employed for protein immobilization.

[0171] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[0172] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine,

arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983], entirely incorporated by reference), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0173] In addition, as will be appreciated by those in the art, labels (including fluorescent, enzymatic, magnetic, radioactive, etc. can all be added to the antibodies (as well as the other compositions of the invention).

Glycosylation

[0174] Another type of covalent modification is alterations in glycosylation. In another embodiment, the antibodies disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to the antibody, wherein said carbohydrate composition differs chemically from that of a parent antibody. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. A preferred form of engineered glycoform is afucosylation, which has been shown to be correlated to an increase in ADCC function, presumably through tighter binding to the FcγRIIIa receptor. In this context, "afucosylation" means that the majority of the antibody produced in the host cells is substantially devoid of fucose, e.g. 90-95-98% of the generated antibodies do not have appreciable fucose as a component of the carbohydrate moiety of the antibody (generally attached at N297 in the Fc region). Defined functionally, afucosylated antibodies generally exhibit at least a 50% or higher affinity to the FcγRIIIa receptor.

[0175] Engineered glycoforms may be generated by a variety of methods known in the art (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, all entirely incorporated by reference; (Potelligent® technology [Biowa, Inc., Princeton, NJ]; GlycoMAb® glycosylation engineering technology [Glycart Biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells, by regulating enzymes involved in the

glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. For example, the "sugar engineered antibody" or "SEA technology" of Seattle Genetics functions by adding modified saccharides that inhibit fucosylation during production; see for example 20090317869, hereby incorporated by reference in its entirety. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an antibody can include an engineered glycoform.

[0176] Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[0177] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0178] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[0179] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has

glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306, both entirely incorporated by reference.

[0180] Removal of carbohydrate moieties present on the starting antibody (e.g. post-translationally) may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131, both entirely incorporated by reference. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350, entirely incorporated by reference. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105, entirely incorporated by reference. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[0181] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in, for example, 2005-2006 PEG Catalog from Nektar Therapeutics (available at the Nektar website) US Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, all entirely incorporated by reference. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037A1, entirely incorporated by reference.

Additional Fc Variants for Additional Functionality

[0182] In addition to pI amino acid variants, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR receptors, altered binding to FcRn receptors, etc.

[0183] Accordingly, the proteins of the invention can include amino acid modifications, including the heterodimerization variants outlined herein, which includes the pI variants

FcγR Variants

[0184] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to Fc□RIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; 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). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L and 299T.

[0185] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

Linkers

[0186] The present invention optionally provides linkers as needed, for example in the addition of additional antigen binding sites, as depicted for example in Figure 2A-2E, where "the other end" of the molecule contains additional antigen binding components. In addition, as outlined below, linkers are optionally also used in antibody drug conjugate (ADC) systems. When used to join the components of the central mAb-Fv constructs, the linker is generally a polypeptide comprising two or more amino acid residues joined by peptide bonds and are used to link one or more of the components of the present invention. Such linker polypeptides are well known in the art (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). A variety of linkers may find use in some embodiments described herein. As will be appreciated by those in the art, there are at least three different linker types used in the present invention.

[0187] "Linker" herein is also referred to as "linker sequence", "spacer", "tethering sequence" or grammatical equivalents thereof. Homo-or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated entirely by reference). A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used. Useful linkers include glycine-serine polymers, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers.

[0188] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example Cκ or Cλ. Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

Antibody-Drug Conjugates

[0189] In some embodiments, the multispecific antibodies of the invention are conjugated with drugs to form antibody-drug conjugates (ADCs). In general, ADCs are used in oncology applications, where the use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents allows for the targeted delivery of the drug moiety to tumors, which can allow higher efficacy, lower toxicity, etc. An overview of this technology is provided in Ducry et al., Bioconjugate Chem., 21:5-13 (2010), Carter et al., Cancer J.

14(3):154 (2008) and Senter, Current Opin. Chem. Biol. 13:235-244 (2009), all of which are hereby incorporated by reference in their entirety.

[0190] Thus the invention provides multispecific antibodies conjugated to drugs. Generally, conjugation is done by covalent attachment to the antibody, as further described below, and generally relies on a linker, often a peptide linkage (which, as described below, may be designed to be sensitive to cleavage by proteases at the target site or not). In addition, as described above, linkage of the linker-drug unit (LU-D) can be done by attachment to cysteines within the antibody. As will be appreciated by those in the art, the number of drug moieties per antibody can change, depending on the conditions of the reaction, and can vary from 1:1 to 10:1 drug:antibody. As will be appreciated by those in the art, the actual number is an average.

[0191] Thus the invention provides multispecific antibodies conjugated to drugs. As described below, the drug of the ADC can be any number of agents, including but not limited to cytotoxic agents such as chemotherapeutic agents, growth inhibitory agents, toxins (for example, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (that is, a radioconjugate) are provided. In other embodiments, the invention further provides methods of using the ADCs.

[0192] Drugs for use in the present invention include cytotoxic drugs, particularly those which are used for cancer therapy. Such drugs include, in general, DNA damaging agents, anti-metabolites, natural products and their analogs. Exemplary classes of cytotoxic agents include the enzyme inhibitors such as dihydrofolate reductase inhibitors, and thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, diynenes, the podophyllotoxins, dolastatins, maytansinoids, differentiation inducers, and taxols.

[0193] Members of these classes include, for example, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosideine, actinomycin, daunorubicin, doxorubicin, mitomycin C, mitomycin A, caminomycin, aminopterin, tallysomycin, podophyllotoxin and podophyllotoxin derivatives such as etoposide or etoposide phosphate, vinblastine, vincristine, vindesine, taxanes including taxol, taxotere retinoic acid, butyric acid, N8-acetyl spermidine, camptothecin, calicheamicin, esperamicin, ene-diynes, duocarmycin A, duocarmycin SA, calicheamicin,

camptothecin, maytansinoids (including DM1), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and maytansinoids (DM4) and their analogues.

[0194] Toxins may be used as antibody-toxin conjugates and include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) J. Nat. Cancer Inst. 92(19):1573-1581; Mandler et al (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). Toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[0195] Conjugates of a multispecific antibody and one or more small molecule toxins, such as a maytansinoids, dolastatins, auristatins, a trichothecene, calicheamicin, and CC1065, and the derivatives of these toxins that have toxin activity, are contemplated.

Maytansinoids

[0196] Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. As described below, drugs may be modified by the incorporation of a functionally active group such as a thiol or amine group for conjugation to the antibody.

[0197] Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared by demethylation using Streptomyces or Actinomyces or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (--OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared by acylation using acyl chlorides) and those having modifications at other positions

[0198] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared by the reaction of maytansinol with H2S or P2S5); C-14-alkoxymethyl(demethoxy/CH2OR) (U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH2OH or CH2OAc) (U.S. Pat. No. 4,450,254) (prepared from Nocardia); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared by the

conversion of maytansinol by Streptomyces); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (isolated from Trewia nudlflora); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

[0199] Of particular use are DM1 (disclosed in US Patent No. 5,208,020, incorporated by reference) and DM4 (disclosed in US Patent No. 7,276,497, incorporated by reference). See also a number of additional maytansinoid derivatives and methods in 5,416,064, WO/01/24763, 7,303,749, 7,601,354, USSN 12/631,508, WO02/098883, 6,441,163, 7,368,565, WO02/16368 and WO04/1033272, all of which are expressly incorporated by reference in their entirety.

[0200] ADCs containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described ADCs comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

[0201] Chari et al., Cancer Research 52:127-131 (1992) describe ADCs in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3x105 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Auristatins and Dolastatins

[0202] In some embodiments, the ADC comprises a multispecific antibody conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Pat. Nos. 5,635,483; 5,780,588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001)

Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al (1998) Antimicrob. Agents Chemother. 42:2961-2965). The dolastatin or auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

[0203] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented Mar. 28, 2004 and described in United States Patent Publication No. 2005/0238648, the disclosure of which is expressly incorporated by reference in its entirety.

[0204] An exemplary auristatin embodiment is MMAE (see US Patent No. 6,884,869 expressly incorporated by reference in its entirety).

[0205] Another exemplary auristatin embodiment is MMAF (see US 2005/0238649, 5,767,237 and 6,124,431, expressly incorporated by reference in their entirety).

[0206] Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):

[0207] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schroder and K. Lubke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al (1998) Anti-Cancer Drug Design 13:243-277; Pettit, G. R., et al. Synthesis, 1996, 719-725; Pettit et al (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863; and Doronina (2003) Nat Biotechnol 21(7):778-784.

Calicheamicin

[0208] In other embodiments, the ADC comprises an antibody of the invention conjugated to one or more calicheamicin molecules. For example, Mylotarg is the first commercial ADC drug and utilizes calicheamicin $\gamma 1$ as the payload (see US Patent No. 4,970,198, incorporated by reference in its entirety). Additional calicheamicin derivatives are described in US Patent Nos. 5,264,586, 5,384,412, 5,550,246, 5,739,116, 5,773,001, 5,767,285 and 5,877,296, all

expressly incorporated by reference. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ1I, α2I, α2I, N-acetyl- γ1I, PSAG and θI1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

Duocarmycins

[0209] CC-1065 (see 4,169,888, incorporated by reference) and duocarmycins are members of a family of antitumor antibiotics utilized in ADCs. These antibiotics appear to work through sequence-selectively alkylating DNA at the N3 of adenine in the minor groove, which initiates a cascade of events that result in apoptosis.

[0210] Important members of the duocarmycins include duocarmycin A (US Patent No. 4,923,990, incorporated by reference) and duocarmycin SA (U.S. Pat. No. 5,101,038, incorporated by reference), and a large number of analogues as described in US Patent Nos. 7,517,903, 7,691,962, 5,101,038; 5,641,780; 5,187,186; 5,070,092; 5,070,092; 5,641,780; 5,101,038; 5,084,468, 5,475,092, 5,585,499, 5,846,545, WO2007/089149, WO2009/017394A1, 5,703,080, 6,989,452, 7,087,600, 7,129,261, 7,498,302, and 7,507,420, all of which are expressly incorporated by reference.

Other Cytotoxic Agents

[0211] Other antitumor agents that can be conjugated to the antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Pat. Nos. 5,053,394, 5,770,710, as well as esperamicins (U.S. Pat. No. 5,877,296).

[0212] Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin,

Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993.

[0213] The present invention further contemplates an ADC formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0214] For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu.

[0215] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as Tc99m or I123, Re186, Re188 and In111 can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate Iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0216] For compositions comprising a plurality of antibodies, the drug loading is represented by p, the average number of drug molecules per Antibody. Drug loading may range from 1 to 20 drugs (D) per Antibody. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Antibody-Drug-Conjugates in terms of p may also be determined.

[0217] In some instances, separation, purification, and characterization of homogeneous Antibody-Drug-conjugates where p is a certain value from Antibody-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In exemplary embodiments, p is 2, 3, 4, 5, 6, 7, or 8 or a fraction thereof.

[0218] The generation of Antibody-drug conjugate compounds can be accomplished by any technique known to the skilled artisan. Briefly, the Antibody-drug conjugate compounds can

include a multispecific antibody as the Antibody unit, a drug, and optionally a linker that joins the drug and the binding agent.

[0219] A number of different reactions are available for covalent attachment of drugs and/or linkers to binding agents. This is can be accomplished by reaction of the amino acid residues of the binding agent, for example, antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. A commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule.

[0220] Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present invention.

[0221] In some embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug under appropriate conditions. In other embodiments, reactive groups are used on the drug and/or the intermediate. The product of the reaction between the drug and the intermediate, or the derivatized drug, is subsequently reacted with an multispecific antibody of the invention under appropriate conditions.

[0222] It will be understood that chemical modifications may also be made to the desired compound in order to make reactions of that compound more convenient for purposes of preparing conjugates of the invention. For example a functional group e.g. amine, hydroxyl, or sulfhydryl, may be appended to the drug at a position which has minimal or an acceptable effect on the activity or other properties of the drug

Linker Units

[0223] Typically, the antibody-drug conjugate compounds comprise a Linker unit between the drug unit and the antibody unit. In some embodiments, the linker is cleavable under intracellular or extracellular conditions, such that cleavage of the linker releases the drug unit from the antibody in the appropriate environment. For example, solid tumors that secrete

certain proteases may serve as the target of the cleavable linker; in other embodiments, it is the intracellular proteases that are utilized. In yet other embodiments, the linker unit is not cleavable and the drug is released, for example, by antibody degradation in lysosomes.

[0224] In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long or more.

[0225] Cleaving agents can include,without limitation, cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Peptidyl linkers that are cleavable by enzymes that are present in CD38-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker (SEQ ID NO: 460)). Other examples of such linkers are described, e.g., in U.S. Pat. No. 6,214,345, incorporated herein by reference in its entirety and for all purposes.

[0226] In some embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker).

[0227] In other embodiments, the cleavable linker is pH-sensitive, that is, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) may be used. (See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929).

[0228] In yet other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Pat. No. 4,880,935.)

[0229] In other embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

[0230] In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety and for all purposes).

[0231] In many embodiments, the linker is self-immolative. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. See for example, WO 2007059404A2, WO06110476A2, WO05112919A2, WO2010/062171, WO09/017394, WO07/089149, WO 07/018431, WO04/043493 and WO02/083180, which are directed to drug-cleavable substrate conjugates where the drug and cleavable substrate are optionally linked through a self-immolative linker and which are all expressly incorporated by reference.

[0232] Often the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, 15%, 10%, 5%, 3%, or no more than about 1% of the linkers, in a sample of antibody-drug conjugate compound, are cleaved when the antibody-drug conjugate compound presents in an extracellular environment (for example, in plasma).

[0233] Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the antibody-drug conjugate compound for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free drug present in the plasma.

[0234] In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent (that is, in the milieu of the linker-therapeutic agent moiety of the antibody-drug conjugate compound as described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the auristatin compound and the multispecific antibodies of the invention.

[0235] A variety of exemplary linkers that can be used with the present compositions and methods are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

Drug Loading

[0236] Drug loading is represented by p and is the average number of Drug moieties per antibody in a molecule. Drug loading ("p") may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more moieties (D) per antibody, although frequently the average number is a fraction or a decimal. Generally, drug loading of from 1 to 4 is frequently useful, and from 1 to 2 is also useful. ADCs of the invention include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy and, ELISA assay.

[0237] The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as electrophoresis.

[0238] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p>5, may cause

aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the drug loading for an ADC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5. See US 2005-0238649 A1 (herein incorporated by reference in its entirety).

[0239] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0240] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachements (such as thioMab or thioFab prepared as disclosed herein and in WO2006/034488 (herein incorporated by reference in its entirety)).

[0241] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography.

[0242] In some embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Methods of Determining Cytotoxic Effect of ADCs

[0243] Methods of determining whether a Drug or Antibody-Drug conjugate exerts a cytostatic and/or cytotoxic effect on a cell are known. Generally, the cytotoxic or cytostatic activity of an Antibody Drug conjugate can be measured by: exposing mammalian cells expressing a target protein of the Antibody Drug conjugate in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays can be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the Antibody Drug conjugate.

[0244] For determining whether an Antibody Drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μCi of 3H-thymidine during the final 8 hours of the 72-hour period. The incorporation of 3H-thymidine into cells of the culture is measured in the presence and absence of the Antibody Drug conjugate.

[0245] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that an Antibody Drug conjugate is useful in the treatment of cancers.

[0246] Cell viability can be measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (see, e.g., Page et al., 1993, Intl. J. Oncology 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan et al., 1990, J. Natl. Cancer Inst. 82:1107-12).

[0247] Alternatively, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, J. Immunol. Methods 65:55-63).

[0248] Apoptosis can be quantitated by measuring, for example, DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica, 1999, no. 2, pp. 34-37 (Roche Molecular Biochemicals).

[0249] Apoptosis can also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al. eds., 1992, pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[0250] The presence of apoptotic cells can be measured in both the attached and "floating" compartments of the cultures. For example, both compartments can be collected by removing the supernatant, trypsinizing the attached cells, combining the preparations following a centrifugation wash step (e.g., 10 minutes at 2000 rpm), and detecting apoptosis (e.g., by measuring DNA fragmentation). (See, e.g., Piazza et al., 1995, Cancer Research 55:3110-16).

[0251] In vivo, the effect of a therapeutic composition of the multispecific antibody of the invention can be evaluated in a suitable animal model. For example, xenogenic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein et al., 1997, Nature Medicine 3: 402-408). Efficacy can be measured using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[0252] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate

buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

Antibody Compositions for In Vivo Administration

[0253] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0254] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide antibodies with other specifcities. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or small molecule antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0255] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0256] The formulations to be used for in vivo administration should be sterile, or nearly so. This is readily accomplished by filtration through sterile filtration membranes.

[0257] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0258] When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37oC, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Administrative modalities

[0259] The antibodies and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as 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. Intravenous or subcutaneous administration of the antibody is preferred.

Treatment modalities

[0260] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended

an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[0261] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[0262] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[0263] Thus for B cell tumors, the subject may experience a decrease in the so-called B symptoms, i.e., night sweats, fever, weight loss, and/or urticaria. For pre-malignant conditions, therapy with an multispecific therapeutic agent may block and/or prolong the time before development of a related malignant condition, for example, development of multiple myeloma in subjects suffering from monoclonal gammopathy of undetermined significance (MGUS).

[0264] An improvement in the disease may be characterized as a complete response. By "complete response" is intended an absence of clinically detectable disease with normalization of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF) or abnormal monoclonal protein in the case of myeloma.

[0265] Such a response may persist for at least 4 to 8 weeks, or sometimes 6 to 8 weeks, following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By "partial response" is intended at least about a 50% decrease in all measurable tumor burden (i.e., the number of malignant cells present in the subject, or the measured bulk of tumor masses or the

quantity of abnormal monoclonal protein) in the absence of new lesions, which may persist for 4 to 8 weeks, or 6 to 8 weeks.

[0266] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[0267] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[0268] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[0269] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0270] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0271] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the

particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0272] The efficient dosages and the dosage regimens for the multispecific antibodies used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[0273] An exemplary, non-limiting range for a therapeutically effective amount of an multispecific antibody used in the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, or about 3 mg/kg. In another embodiment, he antibody is administered in a dose of 1 mg/kg or more, such as a dose of from 1 to 20 mg/kg, e.g. a dose of from 5 to 20 mg/kg, e.g. a dose of 8 mg/kg.

[0274] A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0275] In one embodiment, the multispecific antibody is administered by infusion in a weekly dosage of from 10 to 500 mg/kg such as of from 200 to 400 mg/kg Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours.

[0276] In one embodiment, the multispecific antibody is administered by slow continuous infusion over a long period, such as more than 24 hours, if required to reduce side effects including toxicity.

[0277] In one embodiment the multispecific antibody is administered in a weekly dosage of from 250 mg to 2000 mg, such as for example 300 mg, 500 mg, 700 mg, 1000 mg, 1500 mg or 2000 mg, for up to 8 times, such as from 4 to 6 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. The dosage may be determined or adjusted by measuring the amount of compound of the present invention in the blood upon administration by for instance taking

out a biological sample and using anti-idiotypic antibodies which target the antigen binding region of the multispecific antibody.

[0278] In a further embodiment, the multispecific antibody is administered once weekly for 2 to 12 weeks, such as for 3 to 10 weeks, such as for 4 to 8 weeks.

[0279] In one embodiment, the multispecific antibody is administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

[0280] In one embodiment, the multispecific antibody is administered by a regimen including one infusion of an multispecific antibody followed by an infusion of an multispecific antibody conjugated to a radioisotope. The regimen may be repeated, e.g., 7 to 9 days later.

[0281] As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of an antibody in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

[0282] In some embodiments the multispecific antibody molecule thereof is used in combination with one or more additional therapeutic agents, e.g. a chemotherapeutic agent. Non-limiting examples of DNA damaging chemotherapeutic agents include topoisomerase I inhibitors (e.g., irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (e.g., etoposide, teniposide, and daunorubicin); alkylating agents (e.g., melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, mitomycin C, and cyclophosphamide); DNA intercalators (e.g., cisplatin, oxaliplatin, and carboplatin); DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (e.g., 5-fluorouracil, capecitibine, gemcitabine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea).

[0283] Chemotherapeutic agents that disrupt cell replication include: paclitaxel, docetaxel, and related analogs; vincristine, vinblastin, and related analogs; thalidomide, lenalidomide,

and related analogs (e.g., CC-5013 and CC-4047); protein tyrosine kinase inhibitors (e.g., imatinib mesylate and gefitinib); proteasome inhibitors (e.g., bortezomib); NF-κB inhibitors, including inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

[0284] In some embodiments, the antibodies of the invention can be used prior to, concurrent with, or after treatment with Velcade® (bortezomib).

[0285] All cited references are herein expressly incorporated by reference in their entirety.

[0286] 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.

Examples

[0287] 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. For all constant region 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 Service, National Institutes of Health, Bethesda, entirely incorporated by reference). 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.
EXAMPLE 1. Prototype "triple F" bispecific antibody.

[0288] The present invention describes novel immunoglobulin compositions that co-engage a first and second antigen. One heavy chain of the antibody contains a single chain Fv ("scFv", as defined below) and the other heavy chain is a "regular" Fab format, comprising a variable heavy chain and a light chain (see Fig. 1). This structure is sometimes referred to herein as "triple F" format (scFv-Fab-Fc). The two chains are brought together by the dimeric Fc region (see Fig. 2). The Fc region can be modified by amino acid substitution to allow for efficient purification of the "triple F" heterodimer. Further, the Fc region can be

modified by amino acid substitution to promote the formation of the "triple F" heterodimer. Examples of Fc substitutions are described more fully below.

[0289] Fc substitutions can be included in the "triple F" format to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers. An example of this is in the inclusion of Fc substitutions that alter the isoelectric point (pI) of each monomer so that such that each monomer has a different pI. In this case the desired "triple F" heterodimer will have a different pI than that of the undesired dual scFv-Fc and mAb homodimers, thus facilitating isoelectric purification of the "triple F" heterodimer (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating dual scFv-Fc and mAb homodimers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns). See Fig. 3 for a list of substitutions that can be made in Fc monomer 1 and Fc monomer 2 to allow for efficient purification of the desired "triple F" heterodimer.

[0290] Fc substitutions can be included in the "triple F" format to "skew" the formation toward the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers. For example, see Fig. 4 for a list of substitutions that can be made in Fc monomer 1 and Fc monomer 2 to "skew" production toward the "triple F" heterodimer. Amino acid subsitutions listed in Fig. 3 and Fig. 4 can be combined, leading to an increased yield of "triple F" heterodimer that can be easily purified away from any contaminating dual scFv-Fc and mAb homodimers.

[0291] After optimization of an scFv domain for inclusion in the "triple F" format, an optimized scFv domain can be coupled with a variety of standard antibody heavy chains in a convenient fashion. For example, an anti-CD3 scFv for recruiting T cell cytotoxicity can be coupled with a variety of anti-tumor antigen antibody heavy chains (e.g., those binding CD5, CD20, CD30, CD40, CD33, CD38, EGFR, EpCAM, Her2, HM1.24, or other tumor antigen). Further examples of optimized scFv domains that can be conveniently coupled with standard antibody heavy chains include anti-CD16 scFv for natural killer cell cytotoxicity; anti-CD32b scFv for inhibitory activity (here the coupled antibody heavy chain would bind, e.g., CD19, CD40, CD79a, CD79b, or other immune receptors); and anti-transferrin receptor scFv, anti-insulin receptor, or anti-LRP1 for transport across the blood-brain barrier.

EXAMPLE 2. Multi-specific antibodies derived from the "triple F" format.

[0292] Multi-specific antibodies can be constructed by attaching additional scFv or Fab domains that bind a third antigen to the C-terminus of one of the "triple F" heavy chains. See Fig. 5 for examples. Alternatively, the C-terminal scFv or Fab may bind the first or second antigen, thus conferring bivalency and an increase in overall binding affinity for that antigen.

[0293] Multi-specific antibodies can also be constructed by coupling the scFv-Fc heavy chain of the "triple F" format may with rearranged antibody heavy chains as depicted in Fig. 6. Such rearranged heavy chains may include an additional Fv region that binds a third antigen or an additional Fv region that binds the first antigen or second antigen, thus conferring bivalency and an increase in overall binding affinity for that antigen.

EXAMPLE 3. Anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific.

[0294] Amino acid sequences for anti-CD19 Fab x anti-CD3 scFv "triple F" bispecifics are listed in Fig. 7. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined. Amino acid sequences for preferred humanized anti-CD3 variable regions are listed in Fig. 8 (with CDRs underlined). Some examples of expression and purification of the desired "triple F" species and its bioactivity are given below.

[0295] The production of XENP11874, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv, is outlined in Fig. 9. In Fig. 9A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 9B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 9C).

[0296] XENP11874, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11874 to potently recruit T cells for B cell depletion is shown in Fig. 10.

[0297] The production of XENP11924, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv, is outlined in Fig. 11. In Fig. 11A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 11B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 11C).

[0298] XENP11924, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11924 to potently recruit T cells for the killing of the Raji tumor cell line is shown in Fig. 12.

EXAMPLE 4. Anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific.

[0299] Amino acid sequences for anti-CD38 Fab x anti-CD3 scFv "triple F" bispecifics are listed in Fig. 13. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined. Some examples of expression and purification of the desired "triple F" species and its bioactivity are given below.

[0300] The production of XENP11925, a "triple F" bispecific with an anti-CD38 Fab and anti-CD3 scFv, is outlined in Fig. 14. In Fig. 14A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 14B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 14C).

[0301] XENP11925, anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11925 to potently recruit T cells for the killing of the RPMI8226 tumor cell line is shown in Fig. 15.

CLAIMS

We claim:

1. A heterodimeric antibody comprising:
   a) a first heavy chain comprising:
      i) a first Fc domain; and
      ii) a single chain Fv region that binds a first antigen; and
   b) a second heavy chain comprising:
      i) a second Fc domain;
      ii) a first variable heavy chain; and
      iii) a first variable light chain;

wherein said first and second Fc domains are different.

2. A heterodimeric antibody according to claim 1 wherein the pIs of said first heavy chain and said second heavy chain are at least 0.5 logs apart.

3. A heterodimer antibody according to claim 1 or 2 further comprising an additional antigen binding domain.

4. A heterodimeric antibody according to claim 3 wherein said additional antigen binding domain is selected from the group consisting of an scFv and a Fab.

5. A heterodimeric antibody according to any of claims 1 to 4 wherein said scFv region binds to CD3 and said first heavy and light chains form a domain that binds to an antigen selected from the group consisting of CD5, CD20, CD30, CD33, CD38, CD40, EGFR, EpCAM, Her2, HM1.24.

6. A heterodimeric antibody according to any one of claims 1 -5 wherein said antibody has a structure selected from Figure 1B, 2A, 2B, 2C, 2D, 2D 3A, 3B, 3C, 3D and 3E.

7. A heterodimeric antibody according to any of claims 1-6 wherein the first Fc domain and said second Fc domain comprises a set of corresponding variants selected from Figure 4.

8. A heterodimeric antibody according to any of claims 1-7 wherein the first Fc domain and said second Fc domain comprises a set of corresponding variants selected from Figure 5.

9. A heterodimeric antibody according to any of claims 1-8 wherein the first Fc domain and said second Fc domain comprises a set of corresponding variants selected from Figure 6.

10. A heterodimeric antibody according to any previous claim wherein the pI substitutions in monomer one are ISO(-): I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL44 and the pI subsitutions in monomer 2 are ISO(+RR): Q196K/I199T/P217R/P228R/N276K.

11. A heterodimeric antibody according to any previous claim further comprising a conjugated drug.

12. A composition comprising a heterodimeric antibody according to any previous claim and a carrier.

13. A nucleic acid encoding the first heavy chain of any previous claim.

14. A nucleic acid encoding the second heavy chain of any previous claim.

15. A nucleic acid encoding the second light chain of any previous claim.

16. A nucleic acid encoding the third light chain of any previous claim.

17. An expression vector comprising a nucleic acid according to any of claims 13-16.

18. A host cell comprising a nucleic acid of any of claims 13-16.

19. A host cell comprising the expression vector of claim 17.

20. A method of making an antibody according to any of claims 1-11 comprising
    a) Culturing a host cell comprising:
        i) a nucleic acid encoding said first heavy chain; and
        ii) a nucleic acid encoding said second heavy chain;
    b) under conditions wherein said nucleic acids are expressed.

21. A method of purifying a heterodimeric antibody comprising:
    a) providing an antibody according to any of claims 1-11;
    b) loading said composition onto an ion exchange column; and
    c) collecting the heterodimeric fraction.

22. A method of treating an individual in need thereof comprising administering the composition of any of claims 1-12.

23. A heterodimeric antibody comprising:

a) a first heavy chain comprising:

i) a first Fc domain; and

ii) a single chain Fv region (scFv) that binds CD3; and

b) a second heavy chain comprising:

i) a second Fc domain;

ii) a first variable heavy chain; and

iii) a first variable light chain;

wherein said first and second Fc domains are different.

24. A heterodimeric antibody according to claim 23 wherein said scFv has a sequence comprising a vhCDR1 having SEQ ID NO:411, a vhCDR2 having SEQ ID NO:413, a vhCDR3 having SEQ ID NO:416, a vlCDR1 having SEQ ID NO:420, a vlCDR2 having SEQ ID NO:425 and a vlCDR3 having SEQ ID NO:433.

25. A heterodimeric antibody according to claim 23 wherein said anti-CD3 variable region is an scFv.

26. A heterodimeric antibody according to claim 25 wherein said scFv has a sequence selected from the group consisting of SEQ ID NOs: 4, 8, 16, 20, 32, 40, 52, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 272, 276, 280, 284, 288, 292, 296, 300, 304, 308, 312, 316, 320, 324, 328, 332, 336, 340, 344, 348, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408.

27. A heterodimeric antibody according to claim 23 wherein said anti-CD3 variable region comprises a variable heavy region and a variable light region selected from the group consisting of: SEQ ID NOs: 5 and 6; SEQ ID NOs: 9 and 10; SEQ ID NOs: 13 and 14; SEQ ID NOs: 17 and 18; SEQ ID NOs: 21 and 22; SEQ ID NOs: 25 and 26; SEQ ID NOs: 29 and 30; SEQ ID NOs: 33 and 34; SEQ ID NOs: 37 and 38; SEQ ID NOs: 41 and 42; SEQ ID NOs: 45 and 46; SEQ ID NOs: 49 and 50; SEQ ID NOs: 53 and 54; SEQ ID NOs: 57 and 58; SEQ ID NOs: 61 and 62; SEQ ID NOs: 65 and 66; SEQ ID NOs: 69 and 70; SEQ ID NOs:

73 and 74; SEQ ID NOs: 77 and 78; SEQ ID NOs: 81 and 82; SEQ ID NOs: 85 and 86; SEQ ID NOs: 89 and 90; SEQ ID NOs: 93 and 94; SEQ ID NOs: 97 and 98; SEQ ID NOs: 101 and 102; SEQ ID NOs: 105 and 106;
SEQ ID NOs: 109 and 110; SEQ ID NOs: 113 and 114; SEQ ID NOs: 117 and 118; SEQ ID NOs: 121 and 122; SEQ ID NOs: 125 and 126; SEQ ID NOs: 129 and 130; SEQ ID NOs: 133 and 134; SEQ ID NOs: 137 and 138; SEQ ID NOs: 141 and 142; SEQ ID NOs: 145 and 146; SEQ ID NOs: 149 and 150; SEQ ID NOs: 153 and 154; SEQ ID NOs: 157 and 158; SEQ ID NOs: 161 and 162; SEQ ID NOs: 165 and 166; SEQ ID NOs: 169 and 170; SEQ ID NOs: 173 and 174; SEQ ID NOs: 177 and 178; SEQ ID NOs: 181 and 182; SEQ ID NOs: 185 and 186; SEQ ID NOs: 189 and 190; SEQ ID NOs: 193 and 194; SEQ ID NOs: 197 and 198; SEQ ID NOs: 201 and 202; SEQ ID NOs: 205 and 206; SEQ ID NOs: 209 and 210; SEQ ID NOs: 213 and 214; SEQ ID NOs: 217 and 218; SEQ ID NOs: 221 and 222; SEQ ID NOs: 225 and 226; SEQ ID NOs: 229 and 230; SEQ ID NOs: 233 and 234; SEQ ID NOs: 237 and 238; SEQ ID NOs: 241 and 242; SEQ ID NOs: 245 and 246; SEQ ID NOs: 249 and 250; SEQ ID NOs: 253 and 254; SEQ ID NOs: 257 and 258; SEQ ID NOs: 261 and 262; SEQ ID NOs: 265 and 266; SEQ ID NOs: 269 and 270; SEQ ID NOs: 273 and 274; SEQ ID NOs: 277 and 278; SEQ ID NOs: 281 and 282; SEQ ID NOs: 285 and 286; SEQ ID NOs: 289 and 290; SEQ ID NOs: 293 and 294; SEQ ID NOs: 297 and 298; SEQ ID NOs: 301 and 302; SEQ ID NOs: 305 and 306; SEQ ID NOs: 309 and 310; SEQ ID NOs: 313 and 314; SEQ ID NOs: 317 and 318; SEQ ID NOs: 321 and 322; SEQ ID NOs: 325 and 326; SEQ ID NOs: 329 and 330; SEQ ID NOs: 333 and 334; SEQ ID NOs: 337 and 338; SEQ ID NOs: 341 and 342; SEQ ID NOs: 345 and 346; SEQ ID NOs: 349 and 350; SEQ ID NOs: 353 and 354; SEQ ID NOs: 357 and 358; SEQ ID NOs: 361 and 362; SEQ ID NOs: 365 and 366; SEQ ID NOs: 369 and 370; SEQ ID NOs: 373 and 374; SEQ ID NOs: 377 and 378; SEQ ID NOs: 381 and 382; SEQ ID NOs: 385 and 386; SEQ ID NOs: 389 and 390; SEQ ID NOs: 393 and 394; SEQ ID NOs: 397 and 398; SEQ ID NOs: 401 and 402; SEQ ID NOs: 405 and 406; SEQ ID NOs: 409 and 410.

28. A heterodimeric antibody according to claim 23 wherein said Fc domain has altered binding to a FcγR receptor.

29. A heterodimeric antibody according to claim 28 wherein said Fc domain has altered binding to FcγRIIIa.

30. A heterodimeric antibody according to claim 28 or 29 wherein said altered binding is to FcγRIIIa and said amino acid substitution is selected from the group consisting of 239D, 239E, 236R, 330L, 332D, 332E, 239D/332E, 239D/332E/330L, 267D, 267E, 328F, 328R, 267E/328F, 243L 298A and 236R/328R.

31. A heterodimeric antibody according to any of claims 28 to 30 wherein said Fc domain has altered binding to a FcRn receptor.

32. A heterodimeric antibody according to claim 31 and said amino acid substitution is selected from the group consisting of 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E and 259I/308F/428L.

33. A nucleic acid encoding a composition or heterodimeric antibody of any of the preceding claims.

34. An expression vector comprising a nucleic acid according to claim 33.

35. A host cell comprising a nucleic acid according to claim 33 or 34.

36. A method of making a composition by providing a host cell according to claim 35 and culturing it under conditions wherein said composition is expressed.

37. A method according to claim 36 further comprising running said composition on an ion exchange column.

38. A method of treating a patient in need thereof by administering a composition according to any of claims 1 to 32.

ABSTRACT

The invention provides heterodimeric antibodies comprising a first heavy chain comprising a first Fc domain and a single chain Fv region that binds a first antigen. The heterodimeric antibodies also comprise a second heavy chain comprising a second Fc domain, a first variable heavy chain and a first variable light chain, wherein the first and second Fc domains are different.

Figure 1

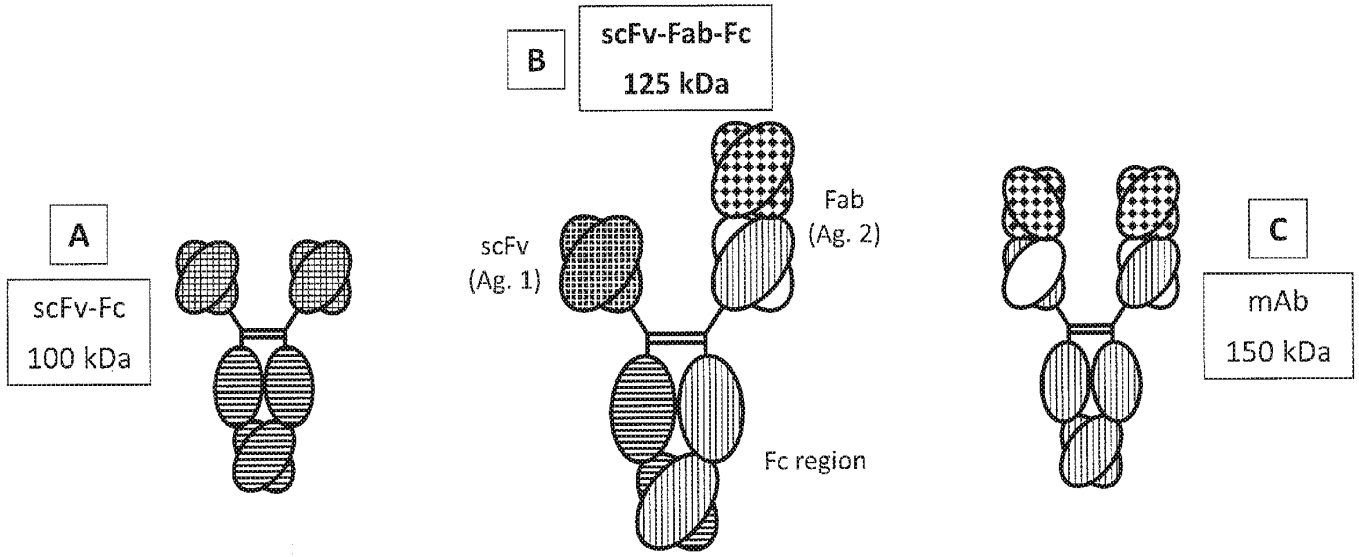
A
scFv-Fc
100 kDa
B
scFv-Fab-Fc
125 kDa
scFv
(Ag. 1)
Fab
(Ag. 2)
Fc region
C
mAb
150 kDa

Figure 2

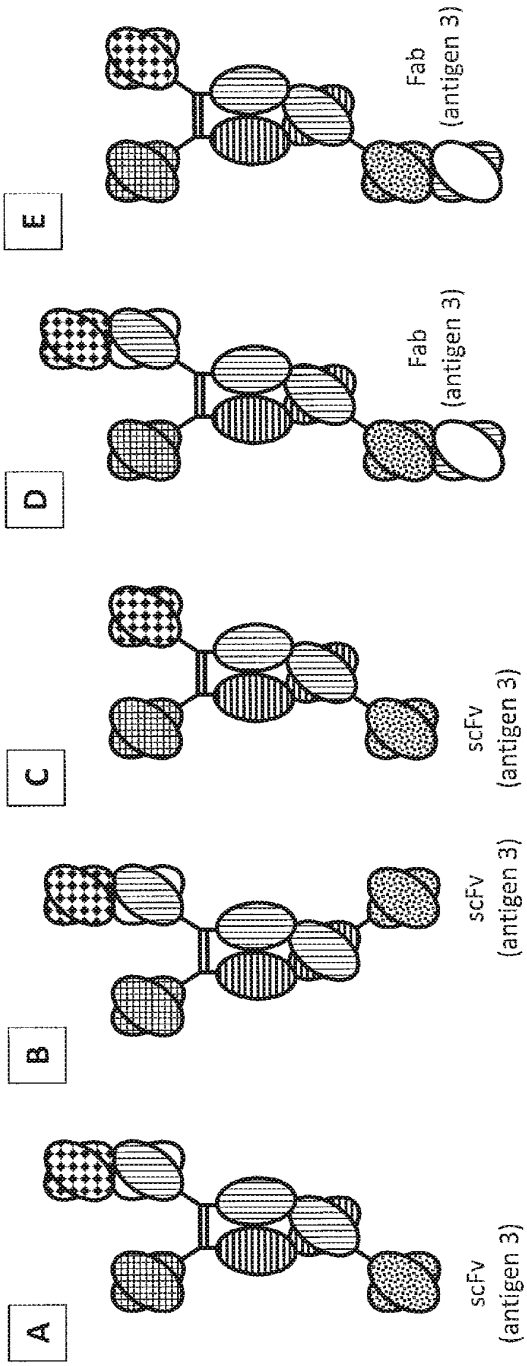
A
scFv
(antigen 3)
B
scFv
(antigen 3)
C
scFv
(antigen 3)
D
Fab
(antigen 3)
E
Fab
(antigen 3)

Figure 3

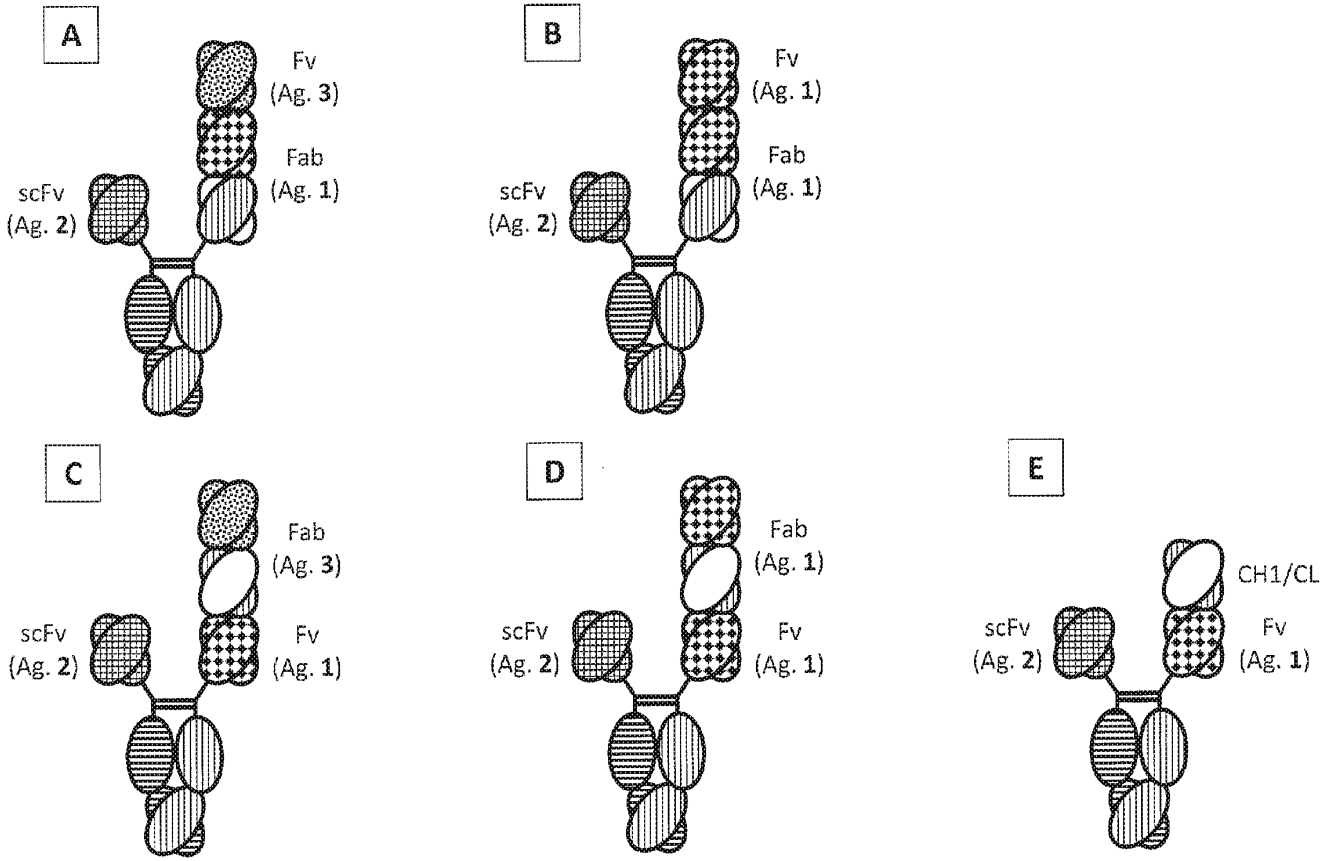
A
Fv
(Ag. 3)
Fab
(Ag. 1)
scFv
(Ag. 2)
B
Fv
(Ag. 1)
Fab
(Ag. 1)
scFv
(Ag. 2)
C
Fab
(Ag. 3)
scFv
(Ag. 2)
Fv
(Ag. 1)
D
Fab
(Ag. 1)
scFv
(Ag. 2)
Fv
(Ag. 1)
E
CH1/CL
scFv
(Ag. 2)
Fv
(Ag. 1)

Figure 4A

Preferred steric variants that favor Fc heterodimerization.

Monomer 1	Monomer 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K
S364F	K370G
S364H	Y349K
S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K
Y349T	S364H
T394F	F405A
Y349T/T394F	S364H/F405A
K370E	T411K
K370E/T411D	T411K
K370E/T411E	K370R/T411K
L368E/K409E	L368K
Y349T/T411E	S364H/D401K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K

Figure 4B

Specifically preferred steric variants that favor Fc heterodimerization.

Variant 1	Variant 2
F405A	T394F
S364D	Y349K
S364E	Y349K
S364H	Y349T
L351K	L351E
D401K	T411E
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
L351K/S364H/D401K	Y349T/L351E/T411E
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K
Y349T	S364H
T394F	F405A
Y349T/T394F	S364H/F405A
K370E	T411K
K370E/T411D	T411K
K370E/T411E	K370R/T411K
L368E/K409E	L368K
Y349T/T411E	S364H/D401K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K

Figure 5A

PREFERRED HETERODIMERIZATION VARIANTS

Fc monomer 1 substitutions	Fc monomer 2 substitutions
Y407T	T366Y
F405A	T394W
T366Y/F405A	T394W/Y407T
Y407A	T366W
T366S/L368A/Y407V	T366W
T366S/L368A/Y407V/Y349C	T366W/S354C
K392D/K409D	E356K/D399K
K370D/K392D/K409D	E356K/E357K/D399K
Y349T	S364H
T394F	F405A
Y349T/T394F	S364H/F405A
K370E	T411K
K370E/T411D	T411K
K370E/T411E	K370R/T411K
L368E/K409E	L368K
Y349T/T411E	S364H/D401K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_ (deletion of K447)	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/N276K
N384S/K392N/V397M/Q419E	N276K
D221E/P228E/L368E	D221R/P228R/K409R
C220E/P228E/L368E	C220R/E224R/P228R/K409R
F405L	K409R
T366I/K392M/T394W	F405A/Y407V
T366V/K409F	L351Y/Y407A
T366A/K392E/K409F/T411E	D399R/S400R/Y407A
Y349T	S364H
T394F	F405A
Y349T/T394F	S364H/F405A
K370E	T411K
K370E/T411D	T411K
K370E/T411E	K370R/T411K
L368E/K409E	L368K
Y349T/T411E	S364H/D401K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R

Figure 5B

Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K

Figure 6

Fc monomer 1 substitutions	Fc monomer 2 substitutions
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/N276K
N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/P217R/P228R/N276K
N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/N276K
N203D/K247Q/R355Q/N384S/K392N/Q419E/K447_	Q196K/P217R/P228R/N276K
N203D/K247Q/R355Q/N384S/K392N/Q419E/K447_	Q196K/N276K

Figure 7A

XENP11874 HC-Fab SEQ ID NO: 441

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKFQGRVTISS
DKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

XENP11874 HC-scFv SEQ ID NO: 442

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQAVV
TQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGKAALT
LSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP11874 LC SEQ ID NO: 443

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPDRFSGSGS
GTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

XENP11924 HC-Fab SEQ ID NO: 444

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKFQGRVTISS
DKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVM
HEALHNHYTQKSLSLSPG

Figure 7B

XENP11924 HC-scFv SEQ ID NO:445

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQAVV
TQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGKAALT
LSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP11924 LC SEQ ID NO: 446

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPDRFSGSGS
GTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

Figure 8

Humanized Anti-CD3 VH with Kabat CDRs underlined SEQ ID NO: 447

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

Humanized Anti-CD3 VL with Kabat CDRs underlined SEQ ID NO: 448

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK
AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 9

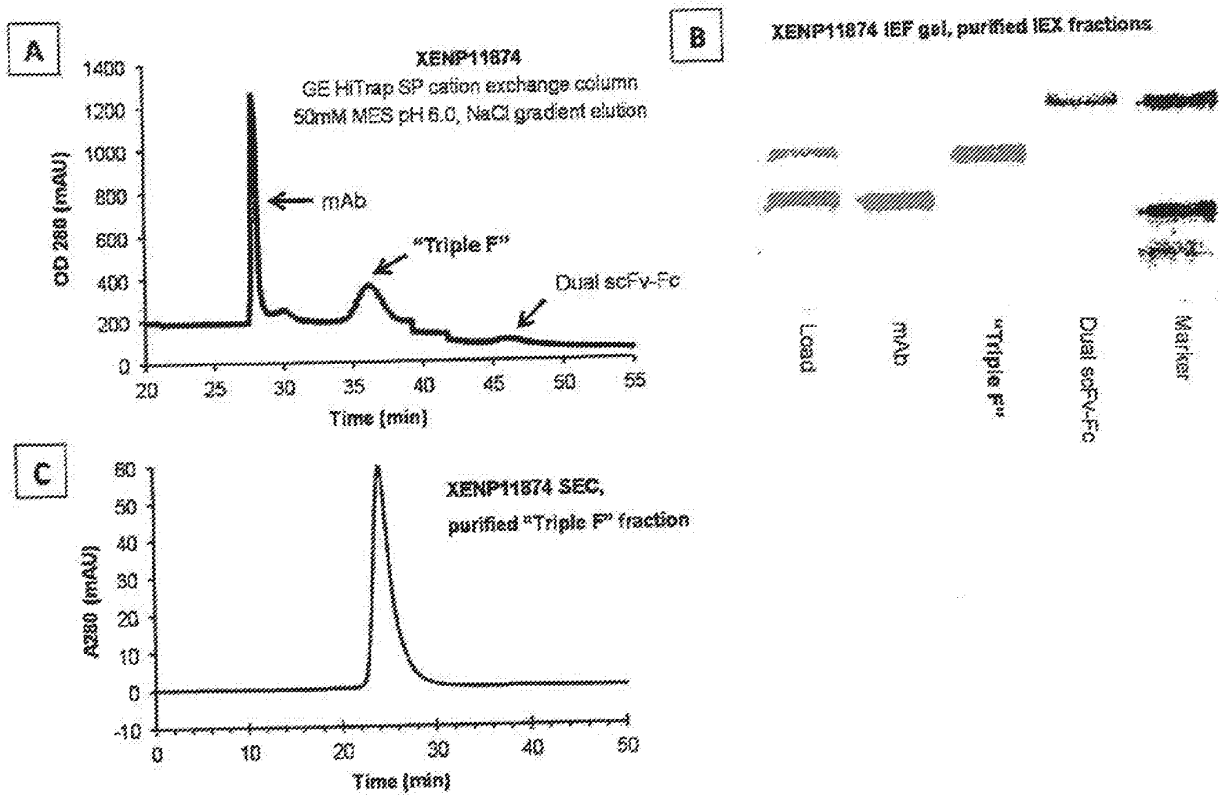
A
XENP11874
GE HiTrap SP cation exchange column
50mM MES pH 6.0, NaCl gradient elution
mAb
"Triple F"
Dual scFv-Fc
OD 280 (mAU)
Time (min)
B
XENP11874 IEF gel, purified IEX fractions
Load
mAb
"Triple F"
Dual scFv-Fc
Marker
C
XENP11874 SEC,
purified "Triple F" fraction
A280 (mAU)
Time (min)

Figure 10

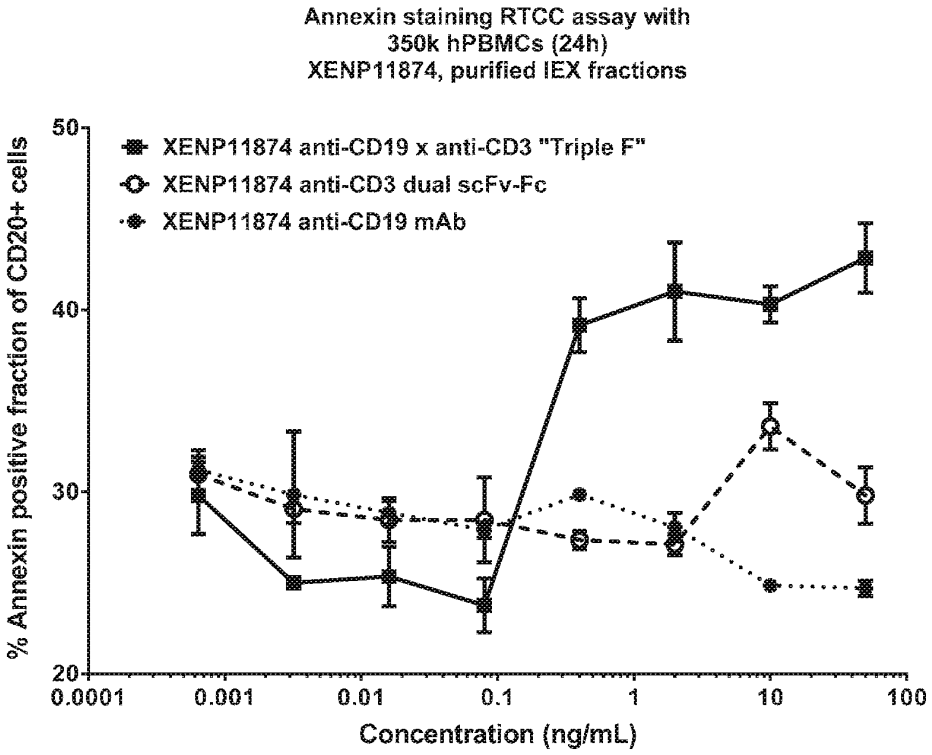
Annexin staining RTCC assay with
350k hPBMCs (24h)
XENP11874, purified IEX fractions
XENP11874 anti-CD19 x anti-CD3 "Triple F"
XENP11874 anti-CD3 dual scFv-Fc
XENP11874 anti-CD19 mAb
% Annexin positive fraction of CD20+ cells
50
40
30
20
0.0001
0.001
0.01
0.1
1
10
100
Concentration (ng/mL)

Figure 11

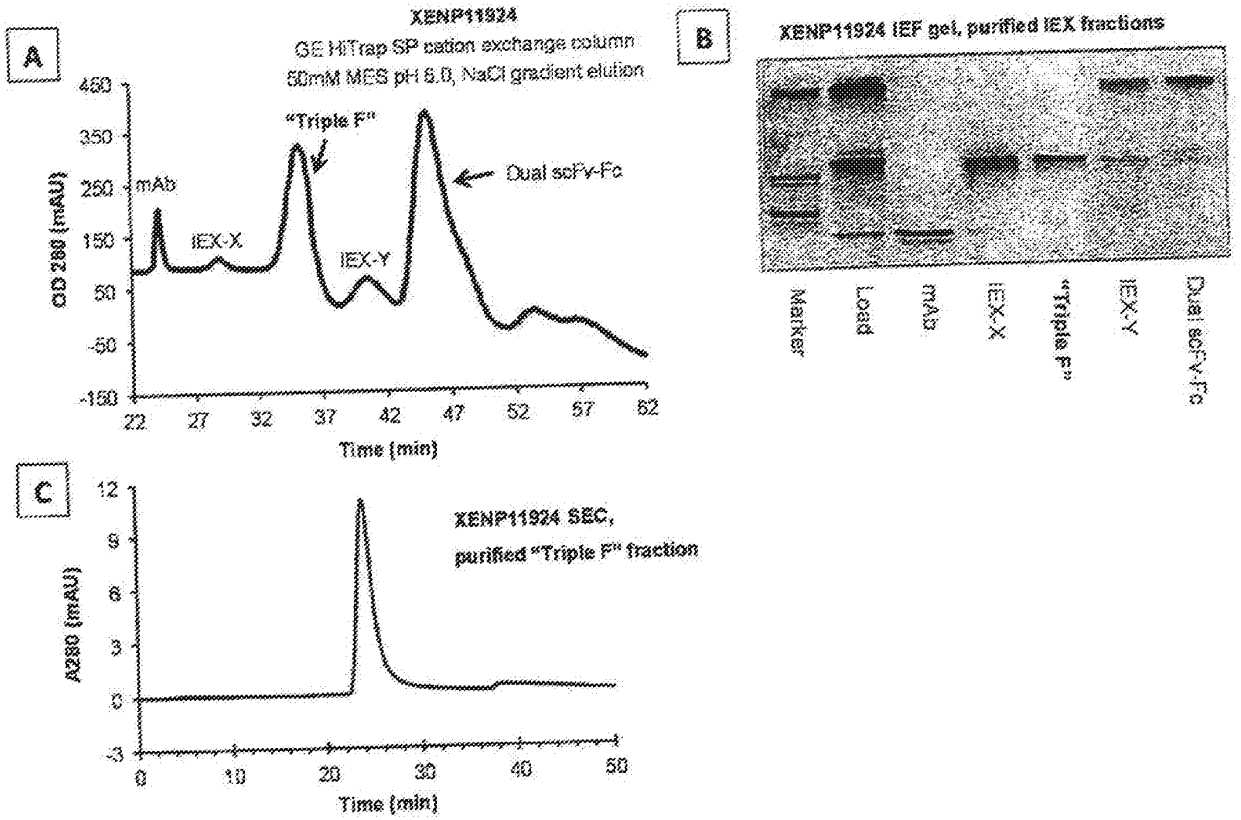
A
XENP11924
GE HiTrap SP cation exchange column
50mM MES pH 6.0, NaCl gradient elution
"Triple F"
Dual scFv-Fc
mAb
IEX-X
IEX-Y
OD 280 (mAU)
Time (min)
B
XENP11924 IEF gel, purified IEX fractions
Marker
Load
mAb
IEX-X
"Triple F"
IEX-Y
Dual scFv-Fc
C
XENP11924 SEC,
purified "Triple F" fraction
A280 (mAU)
Time (min)

Figure 12

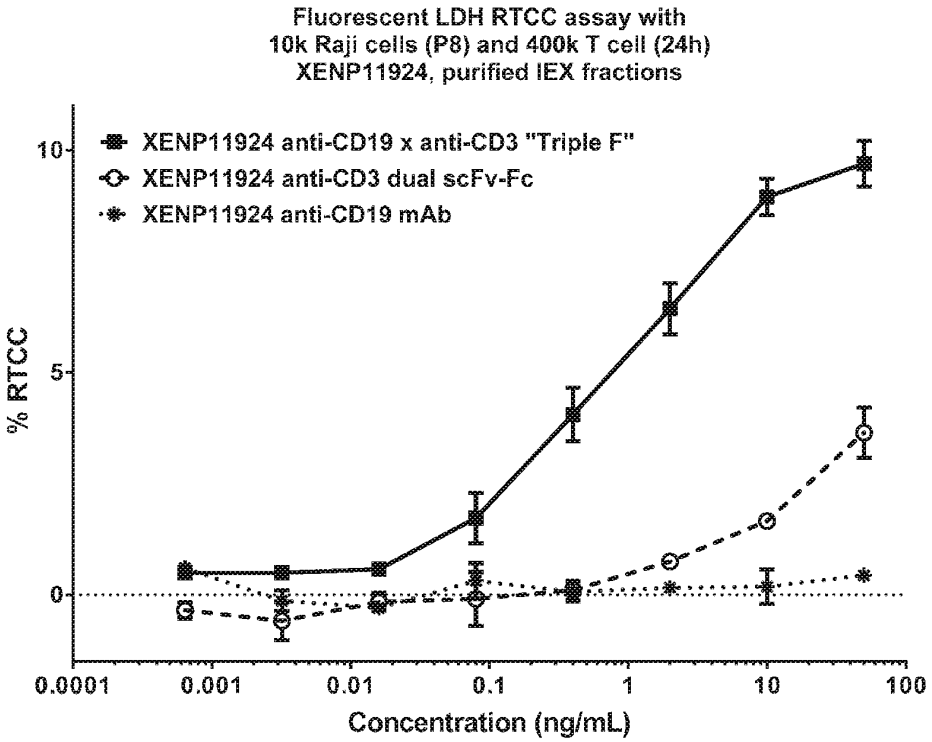
Fluorescent LDH RTCC assay with
10k Raji cells (P8) and 400k T cell (24h)
XENP11924, purified IEX fractions
XENP11924 anti-CD19 x anti-CD3 "Triple F"
XENP11924 anti-CD3 dual scFv-Fc
XENP11924 anti-CD19 mAb
% RTCC
10
5
0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (ng/mL)

Figure 13

XENP11925 HC-Fab SEQ ID NO: 449

EVQLVESGGGLVQPGGSLRLSCAASGFDFSRSWMNWVRQAPGKGLEWVSEINPDSSTINYATSVKGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCARYGNWFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKKVEP
KSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSQEEMTKN
QVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSPG

XENP11925 HC-scFv SEQ ID NO: 450

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQAVV
TQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGKAALT
LSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP11925 LC SEQ ID NO: 451

DIVMTQSPSSLSASVGDRVTITCRASQNVDTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFT
LTISSLQPEDFATYFCQQYDSYPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 14

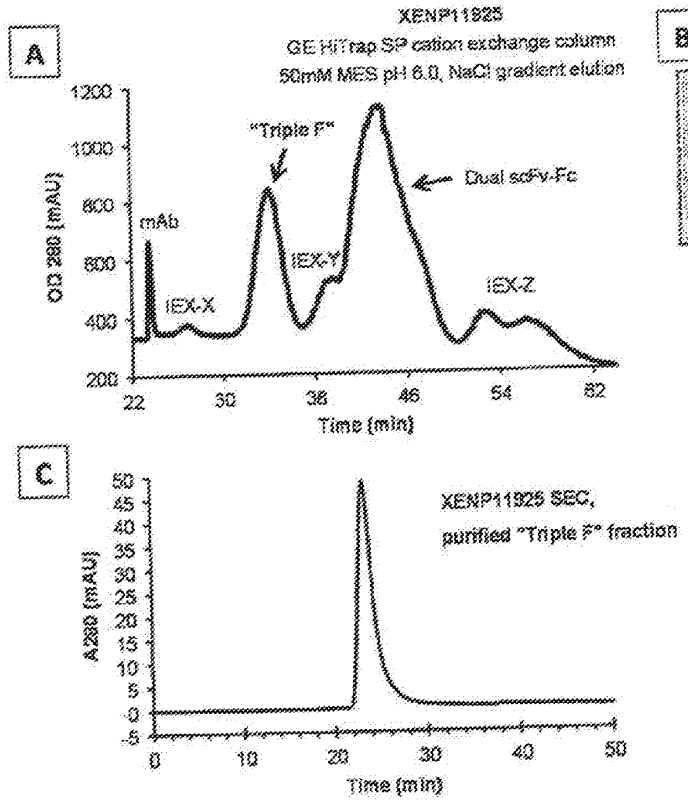
A
XENP11925
GE HiTrap SP cation exchange column
50mM MES pH 6.0, NaCl gradient elution
OD 280 (mAU)
Time (min)
mAb
IEX-X
IEX-Y
IEX-Z
"Triple F"
Dual scFv-Fc
B
XENP11925 IEF gel, purified IEX fractions
Marker
Load
mAb
IEX-X
"Triple F"
IEX-Y
Dual scFv-Fc
IEX-Z
C
XENP11925 SEC,
purified "Triple F" fraction
A280 (mAU)
Time (min)

Figure 15

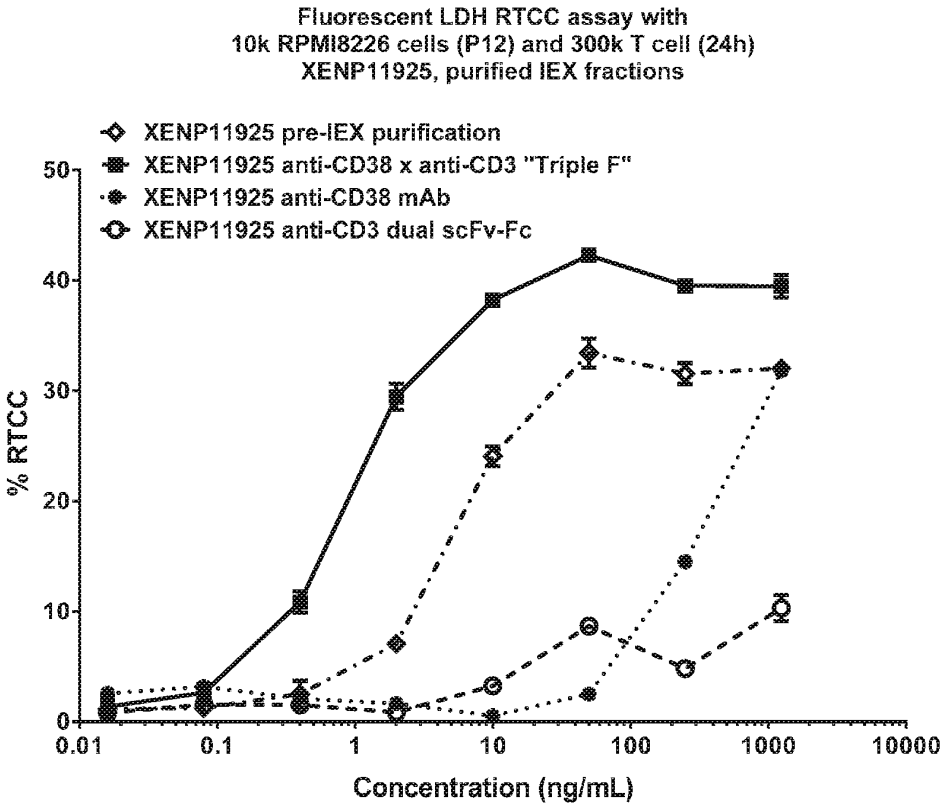
Fluorescent LDH RTCC assay with
10k RPMI8226 cells (P12) and 300k T cell (24h)
XENP11925, purified IEX fractions
XENP11925 pre-IEX purification
XENP11925 anti-CD38 x anti-CD3 "Triple F"
XENP11925 anti-CD38 mAb
XENP11925 anti-CD3 dual scFv-Fc
% RTCC
0
10
20
30
40
50
0.01
0.1
1
10
100
1000
10000
Concentration (ng/mL)

Figure 16A

XENP11924 Heavy Chain HC-Fab SEQ ID NO: 452

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKFQGRVTI
SSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREP
QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSPG

XENP11924 Heavy Chain HC-scFv SEQ ID NO: 453

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP12152 Heavy Chain HC-Fab (T411E) SEQ ID NO: 454

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKFQGRVTI
SSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREP
QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLEVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSPG

XENP12152 Heavy Chain HC-scFv (D401K) SEQ ID NO: 455

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSKGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 16B

XENP12155 Heavy Chain HC-Fab (L368E) SEQ ID NO: 456

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKYNEKFQGRVTI
SSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLRGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKARPAPIEKTISKAKGQPREP
QVYTLPPSQEEMTKNQVSLTCEVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSPG

XENP12155 Heavy Chain HC-scFv (S364K) SEQ ID NO: 457

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLERKSSDKTHTCPRCPAPELLRGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKARPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVKLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP11924, XENP12152, XENP12155 Light Chain SEQ ID NO: 458

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNSGVPDRFSGS
GSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC

Figure 17

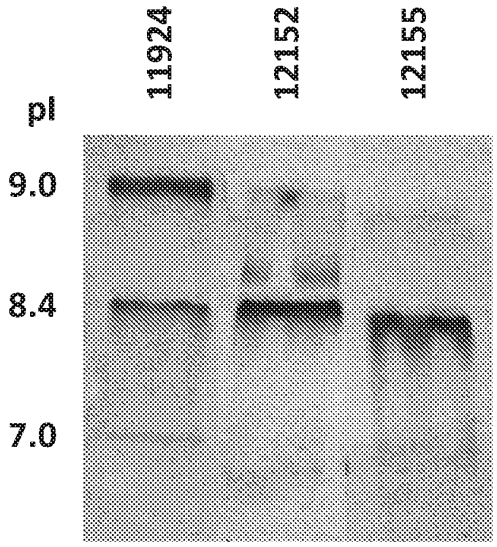

Figure 18

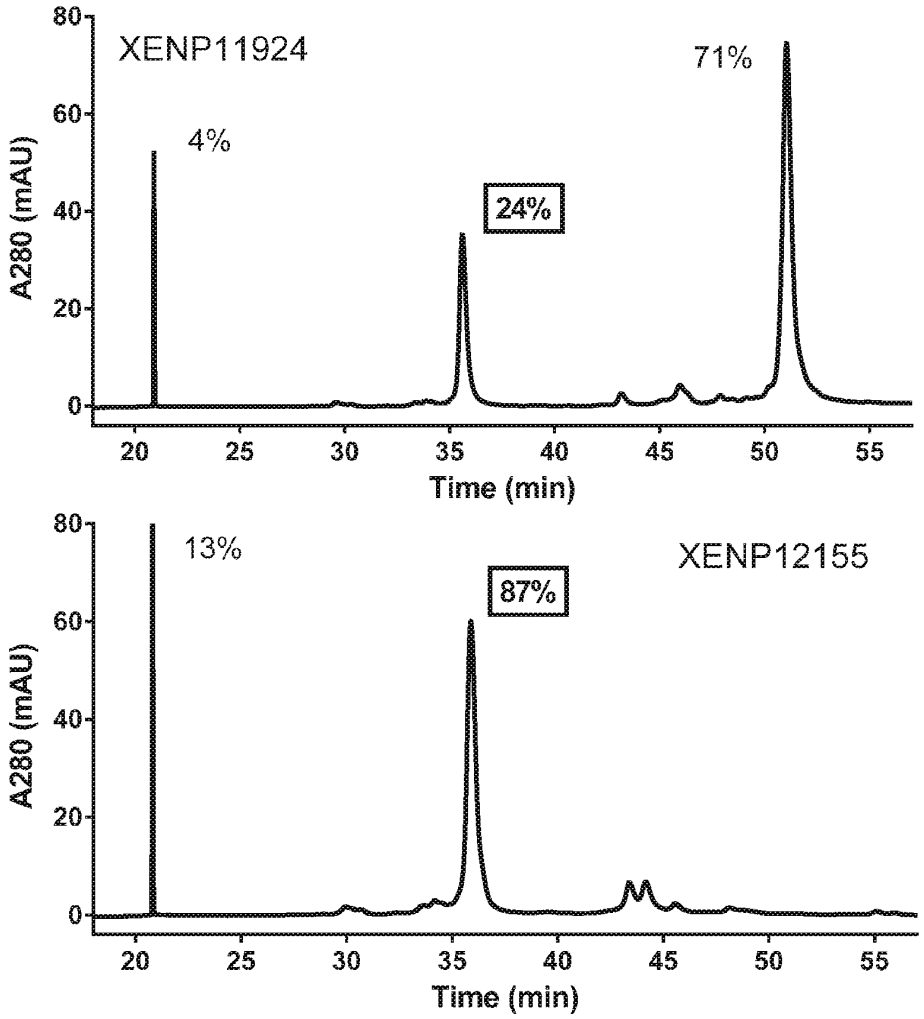
XENP11924
4%
24%
71%
A280 (mAU)
Time (min)
XENP12155
13%
87%
A280 (mAU)
Time (min)

Figure 19

AMINO ACID	pI
Alanine Ala A	6.00
Arginine Arg R	11.15
Asparagine Asn N	5.41
Aspartic acid Asp D	2.77
Cysteine Cys C	5.02
Glutamic acid Glu E	3.22
Glutamine Gln Q	5.65
Glycine Gly G	5.97
Histidine His H	7.47
Isoleucine Ile I	5.94
Leucine Leu L	5.98
Lysine Lys K	9.59
Methionine Met M	5.74
Phenylalanine Phe F	5.48
Proline Pro P	6.30
Serine Ser S	5.68
Threonine Thr T	5.64
Tryptophan Trp W	5.89
Tyrosine Tyr Y	5.66
Valine Val V	5.96

Figure 20A

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP004547	IgG1-WT	2	Ck-WT	112	8.10	122	0	118	0	6			
XENP006384	IgG2-WT	3	Ck-WT	113	7.31	118	-4	118	2	0	27	0	27
NA	IgG3-WT	4	Ck-WT	n/a							22		22
NA	IgG4-WT	5	Ck-WT	n/a							28		28
XENP007349	IgG1/2-HC	6	Ck-WT	114	8.11	120	-2	114	-2	6	11	0	11
XENP005653	IgG1-434S	50	Ck-WT	115	8.1	122	0	116	0	6	1	0	1
XENP006389	IgG2-434S	51	Ck-WT	116	7.31	118	-4	118	2	0	28	0	28
XENP009491	IgG1-CH1-pI(6)	7	Ck-WT	1	6.21	116	-6	128	12	-18	6	0	6
XENP009492	IgG1-WT	2	Ck-pI(6)	8	6.21	116	-6	128	12	-18	0	6	6
XENP009493	IgG1-CH1-pI(6)	192	Ck-pI(6)	117	5.49	110	-12	140	24	-30	6	6	12
XENP009992	IgG1-CH1-pI(6)-434S	52	Ck-pI(6)	117	5.49	110	-12	140	24	-30	7	6	13
XENP009993	IgG1-CH1-pI(6)-428L/434S	53	Ck-pI(6)	117	5.49	110	-12	140	24	-30	8	6	14
XENP010088	IgG1-WT	2	Ck-pI(3)	120	6.58	116	-6	122	6	-6	0	3	3
XENP010089	IgG1-WT	2	Ck-pI(6-DEDE)	121	5.85	116	-6	136	20	-20	0	10	10
XENP010090	IgG2-WT	3	Ck-pI(3)	122	6.16	112	-10	124	8	-12	27	3	30
XENP010091	IgG2-WT	3	Ck-pI(6)	123	5.88	112	-10	130	14	-18	27	6	33
XENP010092	IgG2-WT	3	Ck-pI(6-DEDE)	124	5.58	112	-10	138	22	-26	27	10	37
XENP010093	pI-Iso1	13	Ck-WT	125	6.20	110	-12	122	6	-12	13	0	13
XENP010094	pI-Iso1(NF)	14	Ck-WT	126	6.20	110	-12	122	6	-12	15	0	15
XENP010095	pI-Iso1(NF-VE)	15	Ck-WT	127	6.16	110	-12	122	6	-12	19	0	19
XENP010096	pI-Iso1(NF-VE)	15	Ck-pI(3)	128	5.63	104	-18	128	12	-24	19	3	22
XENP010101	pI-Iso1(NF-VE)	15	Ck-pI(6)	129	5.43	104	-18	134	18	-30	19	6	25
XENP010102	pI-Iso1(NF-VE)	15	Ck-pI(6-DEDE)	130	5.23	104	-18	142	26	-38	19	10	29
XENP010103	pI-Iso1(NF-VE-DEDE)	16	Ck-WT	131	5.79	110	-12	130	14	-20	22	0	22

Figure 20B

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010104	pI-Iso1(NF-VE-DEDE)	16	Ck-pI(3)	132	5.37	104	-18	136	20	-32	22	3	25
XENP010105	pI-Iso1(NF-VE-DEDE)	16	Ck-pI(6)	133	5.22	104	-18	142	26	-38	22	6	28
XENP010106	pI-Iso1(NF-VE-DEDE)	16	Ck-pI(6-DEDE)	134	5.07	104	-18	150	34	-46	22	10	32
XENP010107	IgG1-pI(7)	25	Ck-pI(4)	135	5.31	100	-18	136	20	-36	7	4	11
XENP010108	IgG1-pI(11)	26	Ck-pI(4)	136	4.98	92	-22	144	28	-50	11	4	15
XENP010109	IgG1/2-pI(7)	27	Ck-pI(4)	137	5.36	100	-30	134	18	-48	17	4	21
XENP010110	IgG1/2-pI(11)	28	Ck-pI(4)	145	5.01	92	-22	142	26	-48	21	4	25
XENP010017	IgG1-pI(6)-Neutral-to-DE	56	CK-N152D S156E S202E	138	6.59	122	0	128	12	-8	3	3	6
XENP010018	IgG1-pI(6)-KR-to-Neutral	57	CK-K126Q K145Q K169Q	139	6.58	110	-12	116	0	-8	3	3	6
XENP010019	IgG1-pI(6)-KR-to-DE	58	CK-K126E K145E K169E	140	5.92	110	-12	128	12	-18	3	3	6
XENP010178	IgG-pI-Iso2	19	Ck-WT	141	6.27	110	-12	120	4	-16	28	0	28
XENP010179	IgG-pI-Iso3	22	Ck-WT	142	6.20	110	-12	122	6	-12	19	0	19
XENP010180	IgG-pI-Iso2-4345	62	Ck-WT	143	6.27	110	-12	120	4	-16	29	0	29
XENP010181	IgG-pI-Iso3-4345	63	Ck-WT	144	6.20	110	-12	122	6	-12	20	0	20
XENP010182	IgG-pI-Iso2	19	Ck-pI(4)	145	5.55	102	-20	128	12	-28	28	4	32
XENP010183	IgG-pI-Iso3	22	Ck-pI(4)	145	5.54	102	-20	130	14	-28	19	4	23
XENP010184	IgG-pI-Iso2-4345	62	Ck-pI(4)	145	5.55	102	-20	128	12	-28	29	4	33
XENP010185	IgG-pI-Iso3-4345	63	Ck-pI(4)	145	5.54	102	-20	130	14	-28	20	4	24
XENP010265	IgG-pI-Iso3-222K	70	any light chain	170	6.31	112	-10	122	6	-10	18	0	18
XENP010266	IgG-pI-Iso3-274K	71	any light chain	171	6.31	112	-10	122	6	-10	18	0	18
XENP010267	IgG-pI-Iso3-296Y	72	any light chain	172	6.20	110	-12	122	6	-12	18	0	18
XENP010268	IgG-pI-Iso3-300Y	73	any light chain	173	6.20	110	-12	122	6	-12	18	0	18
XENP010269	IgG-pI-Iso3-309L	74	any light chain	174	6.20	110	-12	122	6	-12	18	0	18

Figure 20C

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010270	IgG-pI-Iso3-339A	75	any light chain	175	6.20	110	-12	122	6	-12	18	0	18
XENP010271	IgG-pI-Iso3-355Q	76	any light chain	176	6.31	112	-10	122	6	-10	18	0	18
XENP010272	IgG-pI-Iso3-384N	77	any light chain	177	6.20	110	-12	122	6	-12	18	0	18
XENP010273	IgG-pI-Iso3-392K	78	any light chain	178	6.31	112	-10	122	6	-10	18	0	18
XENP010274	IgG-pI-Iso3-397V	79	any light chain	179	6.20	110	-12	122	6	-12	18	0	18
XENP010275	IgG-pI-Iso3-419Q	80	any light chain	180	6.31	110	-12	120	4	-10	18	0	18
XENP010276	IgG-pI-Iso3-296Y/300Y	81	any light chain	181	6.20	110	-12	122	6	-12	17	0	17
XENP010277	IgG-pI-Iso3-384N/392K/397V	82	any light chain	182	6.31	112	-10	122	6	-10	16	0	16
XENP010278	IgG-pI-Iso3-137G	83	any light chain	183	6.31	110	-12	120	4	-10	18	0	18
XENP010279	IgG-pI-Iso3-138G	84	any light chain	184	6.20	110	-12	122	6	-12	18	0	18
XENP010280	IgG-pI-Iso3-192S	85	any light chain	185	6.20	110	-12	122	6	-12	18	0	18
XENP010281	IgG-pI-Iso3-193L	86	any light chain	186	6.20	110	-12	122	6	-12	18	0	18
XENP010282	IgG-pI-Iso3-199I	87	any light chain	187	6.20	110	-12	122	6	-12	18	0	18
XENP010283	IgG-pI-Iso3-203N	88	any light chain	188	6.31	110	-12	120	4	-10	18	0	18
XENP010284	IgG-pI-Iso3-214K	89	any light chain	189	6.31	112	-10	122	6	-10	18	0	18
XENP010285	IgG-pI-Iso3-137G/138G	90	any light chain	146	6.31	110	-12	120	4	-10	17	0	17
XENP010286	IgG-pI-Iso3-5L	23	Ck-WT	194	6.20	110	-12	122	6	-12	17	0	17
XENP010287	IgG-pI-Iso3-199I/203N	91	any light chain	195	6.31	110	-12	120	4	-10	17	0	17
XENP010288	IgG-pI-Iso3-214K/222K	92	any light chain	196	6.44	114	-8	122	6	-8	17	0	17
XENP010289	IgG-pI-Iso3-138G/192S/193L	93	any light chain	197	6.20	110	-12	122	6	-12	16	0	16
XENP010290	IgG-PI-Iso3-137G/138G/192S/	94	any light chain	198	6.31	110	-12	120	4	-10	15	0	15

Figure 20D

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010324	IgG-pI-Iso3	22	Ck-Iso(3)	148	5.92	106	-16	124	8	-16	19	3	22
XENP010325	IgG-pI-Iso3	22	Ck-Iso(4)	149	5.83	104	-18	124	8	-20	19	4	23
XENP010326	IgG-pI-Iso3	22	Ck-Iso(5)	150	5.75	104	-18	126	10	-22	19	5	24
XENP010327	IgG-pI-Iso3	22	Ck-Iso(6)	151	5.68	104	-18	128	12	-24	19	6	25
XENP010425	H0L0_IgG2_CH1_IgG1_CH2_CH3	95	any light chain	199	7.30	120	-2	120	4	0	16	0	16
XENP010426	IgG-pI-Iso3-charges-only	24	Ck-WT	152	6.20	110	-12	122	6	-12	9	0	9
XENP010427	IgG-pI-Iso2-charges-only	21	Ck-WT	152	6.27	110	-12	120	4	-10	18	0	18
XENP010428	H0L0_IgG2_CH1_IgG1_Hinge_CH2_CH3	161	any light chain	200	7.67	122	0	120	4	2	9	0	9
XENP010466	IgG-pI-Iso3-SL-4345	98	Ck-WT	152	6.20	110	-12	122	6	-12	18	0	18
XENP010467	IgG-pI-Iso3-SL-428L/4345	97	Ck-WT	152	6.20	110	-12	122	6	-12	19	0	19
XENP010468	H0L0_pI_iso3_S138G/N192S/F193L_N434S	162	any light chain	201	6.20	110	-12	122	6	-12	17	0	17
XENP010469	H0L0_pI_iso3_E137G/S138G/N192S/F193L_N434S	163	any light chain	202	6.31	110	-12	120	4	-10	16	0	16
XENP010470	IgG-pI-Iso2-SL	23	Ck-WT	152	6.27	110	-12	120	4	-10	26	0	26
XENP010471	IgG-pI-Iso2-SL-434S	98	Ck-WT	152	6.27	110	-12	120	4	-10	27	0	27

Figure 20E

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
			Ck-WT	152			-12		6	-12	10	0	10
XENP010472	IgG-pI-Iso3-charges-only-4345	191			6.20	110		122					
			Ck-WT	152			-12		4	-10	19	0	19
XENP010473	IgG-pI-Iso2-charges-only-4345	192			6.27	110		120					
							-2		4	0	17	0	17
XENP010474	H0L0_IgG2_CH1_IgG1_CH2_CH3_M4345	164	any light chain	203	7.30	120		120					
							0		4	2	10	0	10
XENP010475	H0L0_IgG2_CH1_IgG1_Hinge_CH2_CH3_M4345	165	any light chain	204	7.67	122		120					
XENP010476	IgG1_pI(7)-4345	25	Ck-pI(4)	135	5.31	100	-22	136	20	-36	8	4	12
			Ck-pI(4)	135			-22		18	-34	18	4	22
XENP010477	IgG1/2_pI(7)-4345	166			5.36	100		134					
							-12		4	-10	23	0	23
XENP010478	H0L0_pI_iso1/2_charges_only	167	any light chain	205	6.27	110		120					
XENP010511	IgG-pI-Iso3-5L	23	Ck-Iso(3)	148	5.92	106	-16	124	8	-18	17	3	20
XENP010512	IgG-pI-Iso3-5L	23	Ck-Iso(4)	149	5.83	104	-18	124	8	-20	17	4	21
XENP010513	IgG-pI-Iso3-5L	23	Ck-Iso(5)	150	5.75	104	-18	126	10	-22	17	5	22
	IgG-pI-Iso3-5L-	98					-16		8	-18	18	3	21
XENP010517	4345		Ck-Iso(3)	148	5.92	106		124					
	IgG-pI-Iso3-5L-	98					-18		8	-20	18	4	22
XENP010518	4345		Ck-Iso(4)	149	5.83	104		124					
	IgG-pI-Iso3-5L-	98					-18		10	-22	18	5	23
XENP010519	4345		Ck-Iso(5)	150	5.75	104		126					

Figure 20F

XenP#	Name (HC)	SEQ ID NO (HC)	Name (LC)	SEQ ID NO (LC)	Calc. pI	# KR	Delta KR (vs. WT)	# DE	Delta DE (vs. WT)	Charge State	# HC Mutations vs IgG1	# LC Mutations vs IgG1	Total # of Mutations
XENP010520	IgG-pI-Iso3-SL-428L/434S	97	Ck-Iso(3)	148	5.92	106	-16	124	8	-18	19	3	22
XENP010521	IgG-pI-Iso3-SL-428L/434S	97	Ck-Iso(4)	149	5.83	104	-18	124	8	-20	19	4	23
XENP010522	IgG-pI-Iso3-SL-428L/434S	97	Ck-Iso(5)	150	5.75	104	-18	126	10	-22	19	5	24
XENP010525	IgG-pI-Iso3-SL-434S	98	Ck-pI(4)	149	5.54	102	-20	130	14	-28	18	4	22
XENP010526	IgG-pI-Iso3-434S	99	Ck-Iso(5)	150	5.75	104	-18	126	10	-22	20	5	25
XENP010527	IgG-pI-Iso2-SL-434S	66	Ck-Iso(5)	150	5.78	104	-18	124	8	-20	27	5	32

Figure 21A

Variant	# of sub(s)	pI / pI	pI / WT	WT/ WT	avg delta pI
G137E/N203D/K274Q/R355Q/K392N/Q419E/K447_	7	6.43	7.14	8.02	-0.79
G137E/N203D/K274Q/R355Q/K392N/Q419E	6	6.58	7.30	8.02	-0.72
G137E/N203D/K274Q/R355Q/K392N/K447_	6	6.58	7.30	8.02	-0.72
G137E/N203D/K274Q/R355Q/Q419E/K447_	6	6.58	7.30	8.02	-0.72
G137E/N203D/K274Q/K392N/Q419E/K447_	6	6.58	7.30	8.02	-0.72
G137E/N203D/R355Q/K392N/Q419E/K447_	6	6.58	7.30	8.02	-0.72
G137E/K274Q/R355Q/K392N/Q419E/K447_	6	6.58	7.30	8.02	-0.72
N203D/K274Q/R355Q/K392N/Q419E/K447_	6	6.58	7.30	8.02	-0.72
G137E/N203D/K274Q/R355Q/K392N	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/R355Q/Q419E	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/R355Q/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/K392N/Q419E	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/K392N/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/R355Q/K392N/Q419E	5	6.76	7.46	8.02	-0.63
G137E/N203D/R355Q/K392N/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/R355Q/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/K274Q/R355Q/K392N/Q419E	5	6.76	7.46	8.02	-0.63
G137E/K274Q/R355Q/K392N/K447_	5	6.76	7.46	8.02	-0.63
G137E/K274Q/R355Q/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/K274Q/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/R355Q/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
N203D/K274Q/R355Q/K392N/Q419E	5	6.76	7.46	8.02	-0.63
N203D/K274Q/R355Q/K392N/K447_	5	6.76	7.46	8.02	-0.63
N203D/K274Q/R355Q/Q419E/K447_	5	6.76	7.46	8.02	-0.63
N203D/K274Q/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
N203D/R355Q/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
K274Q/R355Q/K392N/Q419E/K447_	5	6.76	7.46	8.02	-0.63
G137E/N203D/K274Q/R355Q	4	7.00	7.61	8.02	-0.51
G137E/N203D/K274Q/K392N	4	7.00	7.61	8.02	-0.51
G137E/N203D/K274Q/Q419E	4	7.00	7.61	8.02	-0.51
G137E/N203D/K274Q/K447_	4	7.00	7.61	8.02	-0.51
G137E/N203D/R355Q/K392N	4	7.00	7.61	8.02	-0.51
G137E/N203D/R355Q/Q419E	4	7.00	7.61	8.02	-0.51
G137E/N203D/R355Q/K447_	4	7.00	7.61	8.02	-0.51
G137E/N203D/K392N/Q419E	4	7.00	7.61	8.02	-0.51
G137E/N203D/K392N/K447_	4	7.00	7.61	8.02	-0.51
G137E/N203D/Q419E/K447_	4	7.00	7.61	8.02	-0.51
G137E/K274Q/R355Q/K392N	4	7.00	7.61	8.02	-0.51
G137E/K274Q/R355Q/Q419E	4	7.00	7.61	8.02	-0.51
G137E/K274Q/R355Q/K447_	4	7.00	7.61	8.02	-0.51
G137E/K274Q/K392N/Q419E	4	7.00	7.61	8.02	-0.51
G137E/K274Q/K392N/K447_	4	7.00	7.61	8.02	-0.51
G137E/K274Q/Q419E/K447_	4	7.00	7.61	8.02	-0.51
G137E/R355Q/K392N/Q419E	4	7.00	7.61	8.02	-0.51
G137E/R355Q/K392N/K447_	4	7.00	7.61	8.02	-0.51
G137E/R355Q/Q419E/K447_	4	7.00	7.61	8.02	-0.51
G137E/K392N/Q419E/K447_	4	7.00	7.61	8.02	-0.51
N203D/K274Q/R355Q/K392N	4	7.00	7.61	8.02	-0.51
N203D/K274Q/R355Q/Q419E	4	7.00	7.61	8.02	-0.51
N203D/K274Q/R355Q/K447_	4	7.00	7.61	8.02	-0.51
N203D/K274Q/K392N/Q419E	4	7.00	7.61	8.02	-0.51

Figure 21B

N203D/K274Q/K392N/K447_	4	7.00	7.61	8.02	-0.51
N203D/K274Q/Q419E/K447_	4	7.00	7.61	8.02	-0.51
N203D/R355Q/K392N/Q419E	4	7.00	7.61	8.02	-0.51
N203D/R355Q/K392N/K447_	4	7.00	7.61	8.02	-0.51
N203D/R355Q/Q419E/K447_	4	7.00	7.61	8.02	-0.51
N203D/K392N/Q419E/K447_	4	7.00	7.61	8.02	-0.51
K274Q/R355Q/K392N/Q419E	4	7.00	7.61	8.02	-0.51
K274Q/R355Q/K392N/K447_	4	7.00	7.61	8.02	-0.51
K274Q/R355Q/Q419E/K447_	4	7.00	7.61	8.02	-0.51
K274Q/K392N/Q419E/K447_	4	7.00	7.61	8.02	-0.51
R355Q/K392N/Q419E/K447_	4	7.00	7.61	8.02	-0.51
G137E/N203D/K274Q	3	7.30	7.74	8.02	-0.36
G137E/N203D/R355Q	3	7.30	7.74	8.02	-0.36
G137E/N203D/K392N	3	7.30	7.74	8.02	-0.36
G137E/N203D/Q419E	3	7.30	7.74	8.02	-0.36
G137E/N203D/K447_	3	7.30	7.74	8.02	-0.36
G137E/K274Q/R355Q	3	7.30	7.74	8.02	-0.36
G137E/K274Q/K392N	3	7.30	7.74	8.02	-0.36
G137E/K274Q/Q419E	3	7.30	7.74	8.02	-0.36
G137E/K274Q/K447_	3	7.30	7.74	8.02	-0.36
G137E/R355Q/K392N	3	7.30	7.74	8.02	-0.36
G137E/R355Q/Q419E	3	7.30	7.74	8.02	-0.36
G137E/R355Q/K447_	3	7.30	7.74	8.02	-0.36
G137E/K392N/Q419E	3	7.30	7.74	8.02	-0.36
G137E/K392N/K447_	3	7.30	7.74	8.02	-0.36
G137E/Q419E/K447_	3	7.30	7.74	8.02	-0.36
N203D/K274Q/R355Q	3	7.30	7.74	8.02	-0.36
N203D/K274Q/K392N	3	7.30	7.74	8.02	-0.36
N203D/K274Q/Q419E	3	7.30	7.74	8.02	-0.36
N203D/K274Q/K447_	3	7.30	7.74	8.02	-0.36
N203D/R355Q/K392N	3	7.30	7.74	8.02	-0.36
N203D/R355Q/Q419E	3	7.30	7.74	8.02	-0.36
N203D/R355Q/K447_	3	7.30	7.74	8.02	-0.36
N203D/K392N/Q419E	3	7.30	7.74	8.02	-0.36
N203D/K392N/K447_	3	7.30	7.74	8.02	-0.36
N203D/Q419E/K447_	3	7.30	7.74	8.02	-0.36
K274Q/R355Q/K392N	3	7.30	7.74	8.02	-0.36
K274Q/R355Q/Q419E	3	7.30	7.74	8.02	-0.36
K274Q/R355Q/K447_	3	7.30	7.74	8.02	-0.36
K274Q/K392N/Q419E	3	7.30	7.74	8.02	-0.36
K274Q/K392N/K447_	3	7.30	7.74	8.02	-0.36
K274Q/Q419E/K447_	3	7.30	7.74	8.02	-0.36
R355Q/K392N/Q419E	3	7.30	7.74	8.02	-0.36
R355Q/K392N/K447_	3	7.30	7.74	8.02	-0.36
R355Q/Q419E/K447_	3	7.30	7.74	8.02	-0.36
K392N/Q419E/K447_	3	7.30	7.74	8.02	-0.36
G137E/N203D	2	7.61	7.85	8.02	-0.21
G137E/K274Q	2	7.61	7.85	8.02	-0.21
G137E/R355Q	2	7.61	7.85	8.02	-0.21
G137E/K392N	2	7.61	7.85	8.02	-0.21
G137E/Q419E	2	7.61	7.85	8.02	-0.21
G137E/K447_	2	7.61	7.85	8.02	-0.21
N203D/K274Q	2	7.61	7.85	8.02	-0.21
N203D/R355Q	2	7.61	7.85	8.02	-0.21
N203D/K392N	2	7.61	7.85	8.02	-0.21
N203D/Q419E	2	7.61	7.85	8.02	-0.21
N203D/K447_	2	7.61	7.85	8.02	-0.21

Figure 21C

K274Q/R355Q	2	7.61	7.85	8.02	-0.21
K274Q/K392N	2	7.61	7.85	8.02	-0.21
K274Q/Q419E	2	7.61	7.85	8.02	-0.21
K274Q/K447_	2	7.61	7.85	8.02	-0.21
R355Q/K392N	2	7.61	7.85	8.02	-0.21
R355Q/Q419E	2	7.61	7.85	8.02	-0.21
R355Q/K447_	2	7.61	7.85	8.02	-0.21
K392N/Q419E	2	7.61	7.85	8.02	-0.21
K392N/K447_	2	7.61	7.85	8.02	-0.21
Q419E/K447_	2	7.61	7.85	8.02	-0.21
G137E	1	7.85	7.94	8.02	-0.09
N203D	1	7.85	7.94	8.02	-0.09
K274Q	1	7.85	7.94	8.02	-0.09
R355Q	1	7.85	7.94	8.02	-0.09
K392N	1	7.85	7.94	8.02	-0.09
Q419E	1	7.85	7.94	8.02	-0.09
K447_	1	7.85	7.94	8.02	-0.09

Figure 22

Variant	# of sub(s)	pI / pI	pI / WT	WT/ WT	avg delta pI
Q196K/P217R/P228R/N276K/H435R	5	8.53	8.32	8.02	0.25
Q196K/P217R/P228R/N276K	4	8.45	8.27	8.02	0.22
Q196K/P217R/P228R/H435R	4	8.46	8.28	8.02	0.22
Q196K/P217R/N276K/H435R	4	8.45	8.27	8.02	0.22
Q196K/P228R/N276K/H435R	4	8.45	8.27	8.02	0.22
P217R/P228R/N276K/H435R	4	8.46	8.28	8.02	0.22
Q196K/P217R/P228R	3	8.37	8.22	8.02	0.17
Q196K/P217R/N276K	3	8.37	8.22	8.02	0.17
Q196K/P217R/H435R	3	8.37	8.22	8.02	0.17
Q196K/P228R/N276K	3	8.37	8.22	8.02	0.17
Q196K/P228R/H435R	3	8.37	8.22	8.02	0.17
Q196K/N276K/H435R	3	8.37	8.22	8.02	0.17
P217R/P228R/N276K	3	8.37	8.22	8.02	0.17
P217R/P228R/H435R	3	8.37	8.22	8.02	0.18
P217R/N276K/H435R	3	8.37	8.22	8.02	0.17
P228R/N276K/H435R	3	8.37	8.22	8.02	0.17
Q196K/P217R	2	8.27	8.16	8.02	0.13
Q196K/P228R	2	8.27	8.16	8.02	0.13
Q196K/N276K	2	8.27	8.16	8.02	0.13
Q196K/H435R	2	8.27	8.16	8.02	0.13
P217R/P228R	2	8.28	8.16	8.02	0.13
P217R/N276K	2	8.27	8.16	8.02	0.13
P217R/H435R	2	8.28	8.16	8.02	0.13
P228R/N276K	2	8.27	8.16	8.02	0.13
P228R/H435R	2	8.28	8.16	8.02	0.13
N276K/H435R	2	8.27	8.16	8.02	0.13
Q196K	1	8.16	8.10	8.02	0.07
P217R	1	8.16	8.10	8.02	0.07
P228R	1	8.16	8.10	8.02	0.07
N276K	1	8.16	8.10	8.02	0.07
H435R	1	8.16	8.10	8.02	0.07

Figure 23A

HETERODIMERIZATION VARIANTS

	IgG1	IgG2	IgG3	IgG4	Variants
196	Q	Q	Q	K	K
199	I	T	T	T	T
203	N	D	N	D	D
217	P	R	L	S	R
220	C	C	P	G	E,R (IgG2)
221	D	_	LGD	_	E,R (IgG1)
222	K	V	T	_	E,R (IgG2)
223	T	_	T	_	D,E,R,K
225	T	_	T	P	D,E,R,K
228	P	P	RCPEPK SCDTPP PCPRCP EPKSCD TPPPCP RCPEPK SCDTPP PCPR	S	D,E,R,K
247	P	P	P	P	Q
276	N	N	K	N	K
340	K	K	K	K	E,Q
345	E	E	E	E	K
	Q	Q	Q	Q	E,K,R
349	Y	Y	Y	Y	A,C,D,E,I,K,S,T,W
350	T	T	T	T	I
351	L	L	L	L	E,K,V,Y
354	S	S	S	S	C
355	R	R	R	Q	E,Q
356	D	E	E	E	K, L, R
357	E	E	E	E	K,R,Q,T
	K	K	K	K	D,E
362	Q	Q	Q	Q	E,K
	S	S	S	S	C,D,E,F,G,H,K,R,T,Y
366	T	T	T	T	A,D,I,K,L,M,S,V,W,Y
	L	L	L	L	A,D,E,K,S all but C,P
370	K	K	K	K	C,D,E,G,R,S,T,V all but C,P
371	G	G	G	G	D
384	N	N	S	N	S
390	N	N	N	N	D,E,K,R
392	K	K	N	K	C,D,E,F,L,M,N
394	T	T	T	T	F,S,V,W,Y

Figure 23B

	IgG1	IgG2	IgG3	IgG4	Variants
395	P	P	P	P	T,V
396	P	P	P	P	T,V
397	V	M	M	V	M,S,T
399	D	D	D	D	all but C,P C,K,R
400	S	S	S	S	A,D,E,K,R
401	D	D	D	D	K,N,R
405	F	F	F	F	L, all but C,P A,F,L,M,S,T,V
407	Y	Y	Y	Y	T,V all but C,P A,L,M,V
409	K	K	K	R	R ,all but C,P D,E,F,K,L,M,V,W
	T	T	T	T	D,E,K,L,N,R,S
419	Q	Q	Q	E	E
439	K	K	K	K	D,E

FIGURE 24

COMBINATIONS OF VARIANTS INTO HETERODIMERIZATION FORMATS

Heterodimerization format	FcRn variants Monomer 1 and/or Monomer 2	Fc variants Monomer 1 and/or Monomer 2	pI variants	Steric variants (including charge pairs)	Combinations (See Legend E)
"bottle opener" scFv-Fab-Fc (Figure 1B)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (Figure 5A)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (scFv-Fab-Fv-scFv; Figure 5A)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (scFv-Fab-Fv-scFv; Figure 5B)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (scFv-Fab-Fv-scFv; Figure 5C)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (scFv-Fab-Fv-Fab; Figure 5D)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)
Trimeric bottle opener (scFv-Fab-Fv-Fab; Figure 5E)	See Legend A	See Legend B	See Legend C	See Legend D	1), 2), 3), 4), 5),6), 7), 8), 9) and 10)

Figure 25A

XENP	VH ID	VL ID	VH Substitutions	VL Substitutions
11502	H1	L1.4		
11794	H1.3	L1.4	L11D	
11795	H1.4	L1.4	L11K	
11796	H1.5	L1.4	L11N	
11801	H1.6	L1.4	L11S	
11802	H1.7	L1.4	R19K	
11803	H1.8	L1.4	N30S	
11804	H1.9	L1.4	N35H	
11805	H1.10	L1.4	N35S	
11806	H1.11	L1.4	P41S	
11807	H1.12	L1.4	Y52CA	
11808	H1.13	L1.4	Y52CA/N54S	
11809	H1.14	L1.4	Y58A	
11810	H1.15	L1.4	D61A	
11811	H1.16	L1.4	D73N	
11812	H1.17	L1.4	D73T	
11813	H1.18	L1.4	L78A	
11814	H1.19	L1.4	A84D	
11815	H1.20	L1.4	A84K	
11816	H1.21	L1.4	A84S	
11817	H1.22	L1.4	V93A	
11818	H1.23	L1.4	V93T	
11819	H1.24	L1.4	N100D	
11820	H1.25	L1.4	N100Q	
11821	H1.26	L1.4	S100AT	
11822	H1.27	L1.4	A101D	
11823	H1.28	L1.4	L108T	
11824	H1.29	L1.30	G44S	G100S
11825	H1	L1.10		E7P
11826	H1	L1.11		T12S
11827	H1	L1.12		G17A
11828	H1	L1.13		G17Q
11829	H1	L1.14		T29S
11830	H1	L1.15		T29S/S30G/N31H
11831	H1	L1.16		G49Y/G50D
11832	H1	L1.17		K53N
11833	H1	L1.18		P56S
11834	H1	L1.19		N52S/K53N/R54K/A55H/P56S/G57W/V58T
11835	H1	L1.20		A60D
11836	H1	L1.21		L66K/L67S
11837	H1	L1.22		L66S/L67S

Figure 25B

XENP	VH ID	VL ID	VH Substitutions	VL Substitutions
11838	H1	L1.23		L75I
11839	H1	L1.24		P80A
11840	H1	L1.25		P80S
11841	H1	L1.26		E85D
11842	H1	L1.27		A89L
11843	H1	L1.28		L95H
11844	H1	L1.29		T105E/V106I/L106AK
11920	H1	L3.1		
11921	H1	L5.1		
11922	H1	L1.31		L75D/E85D/L95H
11923	H1	L1.32		Q38E
12107	H1.38	L1.4	V89T	
12108	H1	L1.33		Q42K
12109	H1	L1.34		Q42T
12110	H1	L1.35		Q42S/A43P
12111	H1	L1.36		A43P
12112	H1	L1.37		A43G
12113	H1	L1.38		A43S
12114	H1	L1.39		A43D
12131	H1.30	L1.4	N30S/N100D	
12132	H1.31	L1.4	N30S/N35S/N100D	
12133	H1.32	L1.4	N30S/Y52CA/N100D	
12134	H1.33	L1.4	N30S/N100D/A101D	
12135	H1.34	L1.4	N30S/N35S/Y52CA/N100D/A101D	
12136	H1.35	L1.4	G10E/L11V/V12K/Q13K/G16E	
12137	H1.36	L1.4	K52BN/Y52C_/N53_/N54G/Y55G/A56S	
12138	H1.37	L1.4	Q39K	
12139	H1.37	L1.32	Q39K	Q38E
12149	H1	L1.40		L75I/E85D/L95H
12213	H1.30	L1.40	N30S/N100D	L75I/E85D/L95H
12214	H1.8	L1.40	N30S	L75I/E85D/L95H
12215	H1.39	L1.40	N30S/Q39K	L75I/E85D/L95H
12216	H1.40	L1.40	N30S/Q39K/N100D	L75I/E85D/L95H
12217	H1.30	L1.41	N30S/N100D	L75I/L95H
12218	H1.8	L1.41	N30S	L75I/L95H
12219	H1.39	L1.41	N30S/Q39K	L75I/L95H
12220	H1.40	L1.41	N30S/Q39K/N100D	L75I/L95H

Figure 25C

XENP	VH ID	VL ID	VH Substitutions	VL Substitutions
12221	H1.30	L1.42	N30S/N100D	L75I/Q42K/L95H
12222	H1.8	L1.42	N30S	L75I/Q42K/L95H
12223	H1.39	L1.42	N30S/Q39K	L75I/Q42K/L95H
12224	H1.40	L1.42	N30S/Q39K/N100D	L75I/Q42K/L95H
12225	H1.30	L1.43	N30S/N100D	L75I/A43S/L95H
12226	H1.8	L1.43	N30S	L75I/A43S/L95H
12227	H1.39	L1.43	N30S/Q39K	L75I/A43S/L95H
12228	H1.40	L1.43	N30S/Q39K/N100D	L75I/A43S/L95H
12229	H1.30	L1.44	N30S/N100D	L75I/Q42K/A43S/L95H
12230	H1.8	L1.44	N30S	L75I/Q42K/A43S/L95H
12231	H1.39	L1.44	N30S/Q39K	L75I/Q42K/A43S/L95H
12232	H1.40	L1.44	N30S/Q39K/N100D	L75I/Q42K/A43S/L95H
12233	H1.30	L1.45	N30S/N100D	L75I/Q42K/E85D/L95H
12234	H1.8	L1.45	N30S	L75I/Q42K/E85D/L95H
12235	H1.39	L1.45	N30S/Q39K	L75I/Q42K/E85D/L95H
12236	H1.40	L1.45	N30S/Q39K/N100D	L75I/Q42K/E85D/L95H
12237	H1.30	L1.46	N30S/N100D	L75I/A43S/E85D/L95H
12238	H1.8	L1.46	N30S	L75I/A43S/E85D/L95H
12239	H1.39	L1.46	N30S/Q39K	L75I/A43S/E85D/L95H
12240	H1.40	L1.46	N30S/Q39K/N100D	L75I/A43S/E85D/L95H
12241	H1.30	L1.47	N30S/N100D	L75I/Q42K/A43S/E85D/L95H
12242	H1.8	L1.47	N30S	L75I/Q42K/A43S/E85D/L95H
12243	H1.39	L1.47	N30S/Q39K	L75I/Q42K/A43S/E85D/L95H
12244	H1.40	L1.47	N30S/Q39K/N100D	L75I/Q42K/A43S/E85D/L95H

Figure 26A

Humanized anti-CD3 heavy chain variable region

SEQ ID NO. 1.

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

Humanized anti-CD3 light chain variable region

SEQ ID NO. 2.

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11502 H1_L1.4

SEQ ID NO. 3.

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 4.

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 5.

EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 6.

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11794 H1.3_L1.4

SEQ ID NO. 7.

EVQLVESGGGDVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 8.

EVQLVESGGGDVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26B

SEQ ID NO. 9.
EVQLVESGGGDVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 10.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11795 H1.4_L1.4

SEQ ID NO. 11.
EVQLVESGGGKVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 12.
EVQLVESGGGKVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 13.
EVQLVESGGGKVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 14.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11796 H1.5_L1.4
SEQ ID NO. 15.
EVQLVESGGGNVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

XENP11796 H1.5_L1.4, cont.
SEQ ID NO. 16.
EVQLVESGGGNVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 17.
EVQLVESGGGNVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

Figure 26C

SEQ ID NO. 18.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11801 H1.6_L1.4
SEQ ID NO. 19.
EVQLVESGGGSVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 20.
EVQLVESGGGSVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 21.
EVQLVESGGGSVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 22.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11802 H1.7_L1.4

SEQ ID NO. 23.
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 24.
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 25.
EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 26.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26D

XENP11803 H1.8_L1.4

SEQ ID NO. 27.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 28.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 29.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 30.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11804 H1.9_L1.4

SEQ ID NO. 31.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMHWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 32.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMHWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 33.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMHWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 34.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26E

XENP11805 H1.10_L1.4

SEQ ID NO. 35.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 36.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 37.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 38.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 39.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 40.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 41.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 42.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26F

XENP11807 H1.12_L1.4

SEQ ID NO. 43.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 44.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 45.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 46.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11808 H1.13_L1.4

SEQ ID NO. 47.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANSYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 48.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANSYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 49.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANSYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 50.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26G

XENP11809 H1.14_L1.4

SEQ ID NO. 51.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATAYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 52.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATAYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 53.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATAYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 54.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11810 H1.15_L1.4

SEQ ID NO. 55.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYAASVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 56.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYAASVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 57.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYAASVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 58.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26H

XENP11811 H1.16_L1.4

SEQ ID NO. 59.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 60.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 61.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 62.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11812 H1.17_L1.4

SEQ ID NO. 63.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDTSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 64.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDTSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 65.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDTSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV

SEQ ID NO. 66.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26I

XENP11813 H1.18_L1.4

SEQ ID NO. 67.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTAYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 68.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTAYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 69.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTAYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 70.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11814 H1.19_L1.4

SEQ ID NO. 71.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRDEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 72.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRDEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 73.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRDEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 74.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26J

XENP11815 H1.20_L1.4

SEQ ID NO. 75.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRKEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 76.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRKEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 77.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRKEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 78.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11816 H1.21_L1.4

SEQ ID NO. 79.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRSEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 80.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRSEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 81.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRSEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 82.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26K

XENP11817 H1.22_L1.4

SEQ ID NO. 83.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 84.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 85.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 86.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 87.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCTRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 88.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCTRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 89.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCTRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 90.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26L

XENP11819 H1.24_L1.4

SEQ ID NO. 91.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 92.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 93.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 94.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 95.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGQSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 96.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGQSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 97.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGQSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 98.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26M

XENP11821 H1.26_L1.4

SEQ ID NO. 99.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNTYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 100.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNTYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 101.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNTYVSWFAYWGQGTLVTVSS

SEQ ID NO. 102.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11822 H1.27_L1.4

SEQ ID NO. 103.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 104.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 105.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFDYWGQGTLVTVSS

SEQ ID NO. 106.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26N

XENP11823 H1.28_L1.4

SEQ ID NO. 107.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 108.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 109.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTVSS

SEQ ID NO. 110.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11824 H1.29_L1.30

SEQ ID NO. 111.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKSLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 112.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKSLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 113.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKSLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 114.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26O

XENP11825 H1_L1.10

SEQ ID NO. 115.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQPPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 116.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQPPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 117.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 118.
QAVVTQPPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11826 H1_L1.11

SEQ ID NO. 119.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 120.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 121.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 122.
QAVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26P

XENP11827 H1_L1.12

SEQ ID NO. 123.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGATVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 124.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGATVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 125.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 126.
QAVVTQEPSLTVSPGATVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11828 H1_L1.13

SEQ ID NO. 127.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGQTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 128.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGQTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 129.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 130.
QAVVTQEPSLTVSPGQTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26Q

XENP11829 H1_L1.14

SEQ ID NO. 131.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 132.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 133.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 134.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11830 H1_L1.15

SEQ ID NO. 135.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 136.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 137.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 138.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26R

XENP11831 H1_L1.16

SEQ ID NO. 139.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIYDTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 140.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIYDTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 141.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 142.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIYDTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11832 H1_L1.17

SEQ ID NO. 143.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 144.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 145.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 146.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26S

XENP11833 H1_L1.18

SEQ ID NO. 147.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRASGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 148.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRASGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 149.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 150.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRASGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11834 H1_L1.19

SEQ ID NO. 151.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTSNKHSWTPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 152.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTSNKHSWTPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 153.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 154.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTSNKHSWTPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26T

XENP11835 H1_L1.20

SEQ ID NO. 155.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPDRFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 156.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPDRFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 157.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 158.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPDRFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP11836 H1_L1.21

SEQ ID NO. 159.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSKSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 160.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSKSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 161.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 162.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSKSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26U

XENP11837 H1_L1.22

SEQ ID NO. 163.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSSSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 164.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSSSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 165.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 166.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSSSG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 167.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 168.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 169.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 170.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26V

XENP11839 H1_L1.24

SEQ ID NO. 171.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQAEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 172.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQAEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 173.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 174.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQAEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 175.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQSEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 176.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQSEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 177.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 178.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQSEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26W

XENP11841 H1_L1.26

SEQ ID NO. 179.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEADYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 180.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEADYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 181.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 182.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEADYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 183.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCLLWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 184.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCLLWYSNLWVFGGGTKLTVL

SEQ ID NO. 185.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 186.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCLLWYSNLWVFGGGTKLTVL

Figure 26X

XENP11843 H1_L1.28

SEQ ID NO. 187.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 188.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 189.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 190.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP11844 H1_L1.29

SEQ ID NO. 191.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLEIKGSHHHHHH

SEQ ID NO. 192.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLEIKGS

SEQ ID NO. 193.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 194.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLEIKGS

Figure 26Y

XENP11920 H1_L3.1

SEQ ID NO. 195.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
EIVMTQSPATLSLSPGERATLSCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSGS
GTDFTLTISSLQPEDFAVYYCALWYSNLWVFGGGTKVEIKGSHHHHHH

SEQ ID NO. 196.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
EIVMTQSPATLSLSPGERATLSCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSGS
GTDFTLTISSLQPEDFAVYYCALWYSNLWVFGGGTKVEIKGS

SEQ ID NO. 197.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 198.
EIVMTQSPATLSLSPGERATLSCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSGS
GTDFTLTISSLQPEDFAVYYCALWYSNLWVFGGGTKVEIKGS

XENP11921 H1_L5.1

SEQ ID NO. 199.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
DIVMTQSPDSLAVSLGERATINCKSSTGAVTTSNYANWVQQKPGQPPKGLIGGTNKRAPGVPDRFSGSGS
GTDFTLTISSLQAEDVAVYYCALWYSNLWVFGGGTKVEIKGSHHHHHH

SEQ ID NO. 200.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
DIVMTQSPDSLAVSLGERATINCKSSTGAVTTSNYANWVQQKPGQPPKGLIGGTNKRAPGVPDRFSGSGS
GTDFTLTISSLQAEDVAVYYCALWYSNLWVFGGGTKVEIKGS

SEQ ID NO. 201.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 202.
DIVMTQSPDSLAVSLGERATINCKSSTGAVTTSNYANWVQQKPGQPPKGLIGGTNKRAPGVPDRFSGSGS
GTDFTLTISSLQAEDVAVYYCALWYSNLWVFGGGTKVEIKGS

Figure 26Z

XENP11922 H1_L1.31

SEQ ID NO. 203.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTDSGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 204.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTDSGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 205.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 206.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTDSGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP11923 H1_L1.32

SEQ ID NO. 207.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 208.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 209.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 210.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26AA

XENP12107 H1.38_L1.4

SEQ ID NO. 211.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTATYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 212.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTATYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 213.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTATYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 214.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 215.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 216.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 217.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 218.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLT

Figure 26BB

XENP12109 H1_L1.34

SEQ ID NO. 219.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGTAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 220.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGTAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 221.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 222.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGTAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 223.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGSPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 224.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGSPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 225.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 226.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGSPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26CC

XENP12111 H1_L1.36

SEQ ID NO. 227.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 228.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 229.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 230.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQPPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP12112 H1_L1.37

SEQ ID NO. 231.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQGPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 232.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQGPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 233.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 234.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQGPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26DD

XENP12113 H1_L1.38

SEQ ID NO. 235.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 236.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 237.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 238.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP12114 H1_L1.39

SEQ ID NO. 239.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQDPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 240.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQDPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 241.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 242.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQDPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26EE

XENP12131 H1.30_L1.4

SEQ ID NO. 243.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 244.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 245.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 246.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 247.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 248.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 249.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 250.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26FF

XENP12133 H1.32_L1.4

SEQ ID NO. 251.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 252.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 253.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 254.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP12134 H1.33_L1.4

SEQ ID NO. 255.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 256.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 257.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSS

SEQ ID NO. 258.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26GG

XENP12135 H1.34_L1.4

SEQ ID NO. 259.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 260.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 261.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTLVTVSS

SEQ ID NO. 262.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP12136 H1.35_L1.4

SEQ ID NO. 263.
EVQLVESGGEVKKPGESLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 264.
EVQLVESGGEVKKPGESLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 265.
EVQLVESGGEVKKPGESLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 266.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26HH

XENP12137 H1.36_L1.4

SEQ ID NO. 267.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSNGGSTYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQA
VVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK
AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 268.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSNGGSTYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSQA
VVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK
AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 269.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSNGGSTYYADSVKGRFTI
SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 270.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

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SEQ ID NO. 271.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 272.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 273.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 274.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

Figure 26II

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SEQ ID NO. 275.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 276.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

SEQ ID NO. 277.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 278.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

XENP12149 H1_L1.40

SEQ ID NO. 279.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 280.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 281.
EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 282.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26JJ

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SEQ ID NO. 283.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 284.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 285.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 286.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12214 H1.8_L1.40

SEQ ID NO. 287.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 288.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 289.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 290.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26KK

XENP12215 H1.39_L1.40

SEQ ID NO. 291.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 292.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 293.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 294.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12216 H1.40_L1.40

SEQ ID NO. 295.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 296.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 297.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 298.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26LL

XENP12217 H1.30_L1.41

SEQ ID NO. 299.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 300.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 301.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 302.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12218 H1.8_L1.41

SEQ ID NO. 303.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 304.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 305.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 306.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26MM

XENP12219 H1.39_L1.41

SEQ ID NO. 307.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 308.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 309.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 310.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12220 H1.40_L1.41

SEQ ID NO. 311.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 312.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 313.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 314.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26NN

XENP12221 H1.30_L1.42

SEQ ID NO. 315.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 316.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 317.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 318.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12222 H1.8_L1.42

SEQ ID NO. 319.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 320.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 321.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 322.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26OO

XENP12223 H1.39_L1.42

SEQ ID NO. 323.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 324.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 325.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 326.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12224 H1.40_L1.42

SEQ ID NO. 327.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 328.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 329.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 330.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26PP

XENP12225 H1.30_L1.43

SEQ ID NO. 331.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 332.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 333.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 334.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12226 H1.8_L1.43

SEQ ID NO. 335.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 336.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 337.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 338.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26QQ

XENP12227 H1.39_L1.43

SEQ ID NO. 339.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 340.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 341.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 342.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12228 H1.40_L1.43

SEQ ID NO. 343.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 344.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 345.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 346.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26RR

XENP12229 H1.30_L1.44

SEQ ID NO. 347.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 348.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 349.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 350.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

XENP12230 H1.8_L1.44

SEQ ID NO. 351.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 352.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 353.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 354.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26SS

XENP12231 H1.39_L1.44

SEQ ID NO. 355.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 356.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 357.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 358.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

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SEQ ID NO. 359.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 360.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 361.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 362.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

Figure 26TT

XENP12233 H1.30_L1.45

SEQ ID NO. 363.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 364.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 365.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 366.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12234 H1.8_L1.45

SEQ ID NO. 367.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 368.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 369.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 370.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26UU

XENP12235 H1.39_L1.45

SEQ ID NO. 371.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 372.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 373.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 374.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12236 H1.40_L1.45

SEQ ID NO. 375.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 376.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 377.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 378.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26VV

XENP12237 H1.30_L1.46

SEQ ID NO. 379.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 380.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 381.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 382.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12238 H1.8_L1.46

SEQ ID NO. 383.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 384.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 385.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 386.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26WW

XENP12239 H1.39_L1.46

SEQ ID NO. 387.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 388.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 389.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 390.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12240 H1.40_L1.46

SEQ ID NO. 391.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 392.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 393.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 394.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26XX

XENP12241 H1.30_L1.47

SEQ ID NO. 395.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 396.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 397.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 398.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12242 H1.8_L1.47

SEQ ID NO. 399.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 400.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 401.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 402.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 26YY

XENP12243 H1.39_L1.47

SEQ ID NO. 403.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 404.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 405.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 406.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

XENP12244 H1.40_L1.47

SEQ ID NO. 407.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVLGSHHHHHH

SEQ ID NO. 408.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGS
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO. 409.
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRF
TISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTLVTVSS

SEQ ID NO. 410.
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLG
GKAALTISGAQPEDEADYYCALWYSNHWVFGGGTKLTVL

Figure 27A

XENP	VH ID	VL ID	Yield (mg/L)	T_m (°C)
11502	H1	L1.4	13.39	62.7
11794	H1.3	L1.4	4.00	62
11795	H1.4	L1.4	18.01	62.5
11796	H1.5	L1.4	0.15	61.5
11801	H1.6	L1.4	2.13	62.5
11802	H1.7	L1.4	14.66	62.25
11803	H1.8	L1.4	1.85	64.125
11804	H1.9	L1.4	20.57	65
11805	H1.10	L1.4	29.45	68.5
11806	H1.11	L1.4	1.71	59.75
11807	H1.12	L1.4	6.27	63.375
11808	H1.13	L1.4	9.70	62.375
11809	H1.14	L1.4	3.22	63
11810	H1.15	L1.4	15.38	62
11811	H1.16	L1.4	3.98	58
11812	H1.17	L1.4	0.16	60
11813	H1.18	L1.4	9.09	61
11814	H1.19	L1.4	2.53	62
11815	H1.20	L1.4	5.29	62.5
11816	H1.21	L1.4	19.89	62
11817	H1.22	L1.4	7.38	59
11818	H1.23	L1.4	7.90	62
11819	H1.24	L1.4	3.10	63.125
11820	H1.25	L1.4	4.57	61.875
11821	H1.26	L1.4	57.12	61.5
11822	H1.27	L1.4	25.53	65.5
11823	H1.28	L1.4	6.66	62.5
11824	H1.29	L1.30	30.48	61
11825	H1	L1.10	18.02	62
11826	H1	L1.11	6.36	62.5
11827	H1	L1.12	19.82	61.75
11828	H1	L1.13	2.22	62
11829	H1	L1.14	20.47	62.5
11830	H1	L1.15	20.63	62
11831	H1	L1.16	34.29	61.75
11832	H1	L1.17	17.89	62.25
11833	H1	L1.18	3.85	60
11834	H1	L1.19	6.02	60.75
11835	H1	L1.20	22.46	62.5
11836	H1	L1.21	8.16	55
11837	H1	L1.22	3.51	56.75
11838	H1	L1.23	15.90	63.5

Figure 27B

XENP	VH ID	VL ID	Yield (mg/L)	T_m (°C)
11839	H1	L1.24	15.94	61.75
11840	H1	L1.25	15.11	61.5
11841	H1	L1.26	7.30	64
11842	H1	L1.27	4.39	57.5
11843	H1	L1.28	15.61	62.875
11844	H1	L1.29	12.74	61.5
11920	H1	L3.1	0.72	52
11921	H1	L5.1	1.88	55.5
11922	H1	L1.31	8.89	58.25
11923	H1	L1.32	5.02	58.25
12107	H1.38	L1.4	19.28	62.5
12108	H1	L1.33	14.77	63
12109	H1	L1.34	10.77	62
12110	H1	L1.35	11.61	62.5
12111	H1	L1.36	17.51	62.5
12112	H1	L1.37	13.42	61
12113	H1	L1.38	13.03	63
12114	H1	L1.39	9.78	55.5
12131	H1.30	L1.4	12.73	65
12132	H1.31	L1.4	31.88	71
12133	H1.32	L1.4	24.00	65.5
12134	H1.33	L1.4	26.84	67.5
12135	H1.34	L1.4	40.40	72.5
12136	H1.35	L1.4	8.34	60.5
12137	H1.36	L1.4	4.30	60
12138	H1.37	L1.4	14.19	64.75
12139	H1.37	L1.32	6.75	63.5
12149	H1	L1.40	58.86	65.5

Figure 28A

XENP	% Improvement from H1_L1.4
11796	125.1
11803	118.0
11804	12.2
11805	16.3
11807	31.6
11808	54.4
11809	40.2
11502	129.3
11819	69.2
11820	98.8
11822	7.6
11838	169.8
11841	95.1
11843	152.5
11794	112.1
11795	241.8
11801	77.1
11802	232.6
11806	93.8
11810	263.9
11811	42.4
11812	5.1
11813	107.4
11814	83.8
11815	173.6
11816	230.7
11817	46.4
11818	33.3
11823	127.3
11825	227.4
11826	137.3
11827	197.1
11828	82.3
11829	225.4
11830	206.7
11832	79.9
11833	35.7
11834	66.9
11835	194.7
11836	191.6

Figure 28B

XENP	% Improvement from H1_L1.4
11837	73.9
11839	212.5
11840	204.0
11842	3.4
11844	112.3
12131	29.0
12132	0.1
12133	10.4
12134	1.0
12136	64.4
12137	0.1
12138	108.9
12108	145.2
12113	112.9
12149	128.7

Figure 29

HUMAN T-cell surface glycoprotein CD3 epsilon chain SEQ ID NO: 459

MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPG
SEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSK
PEDANFYLYLRARVCENCMEMDVMSVATIVIVDICITGGLLLLVYYWSK
NRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPDYEPIRKGQRDLYSGL
NQRRI

Figure 30A

Optimized scFv CD3 variable regions

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP11502 H1_L1.4 SEQ ID NO: 4	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11794 H1.3_L1.4 SEQ ID NO: 8	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11795 H1.4_L1.4 SEQ ID NO: 12	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11796 H1.5_L1.4 SEQ ID NO: 16	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11801 H1.6_L1.4 SEQ ID NO: 20	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11802 H1.7_L1.4 SEQ ID NO: 24	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11803 H1.8_L1.4 SEQ ID NO: 28	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11804 H1.9_L1.4 SEQ ID NO: 32	TYAMH SEQ ID NO: 432	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11805 H1.10_L1.4 SEQ ID NO: 36	TYAMS SEQ ID NO: 412	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11806 H1.11_L1.4 SEQ ID NO: 40	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11807 H1.12_L1.4 SEQ ID NO: 44	TYAMN SEQ ID NO: 411	RIRSKANNYATYYADSVKG SEQ ID NO: 414	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430

Figure 30B

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP11808 H1.13_L1.4 SEQ ID NO: 48	TYAMN SEQ ID NO: 411	RIRSKANSYATYYADSVKG SEQ ID NO: 434	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11809 H1.14_L1.4 SEQ ID NO: 52	TYAMN SEQ ID NO: 411	RIRSKYNNYATAYADSVKG SEQ ID NO: 429	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11810 H1.15_L1.4 SEQ ID NO: 56	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYAASVKG SEQ ID NO: 431	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11811 H1.16_L1.4 SEQ ID NO: 60	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11812 H1.17_L1.4 SEQ ID NO: 64	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11813 H1.18_L1.4 SEQ ID NO: 68	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11814 H1.19_L1.4 SEQ ID NO: 72	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11815 H1.20_L1.4 SEQ ID NO: 76	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11816 H1.21_L1.4 SEQ ID NO: 80	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11817 H1.22_L1.4 SEQ ID NO: 84	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11818 H1.23_L1.4 SEQ ID NO: 88	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11819 H1.24_L1.4 SEQ ID NO: 92	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430

Figure 30C

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP11820 H1.25_L1.4 SEQ ID NO: 96	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGQSYVSWFAY SEQ ID NO: 418	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11821 H1.26_L1.4 SEQ ID NO: 100	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNTYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11822 H1.27_L1.4 SEQ ID NO: 104	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFDY SEQ ID NO: 461	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11823 H1.28_L1.4 SEQ ID NO: 108	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11824 H1.29_L1.30 SEQ ID NO: 112	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11825 H1_L1.10 SEQ ID NO: 116	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11826 H1_L1.11 SEQ ID NO: 120	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11827 H1_L1.12 SEQ ID NO: 124	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11828 H1_L1.13 SEQ ID NO: 128	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11829 H1_L1.14 SEQ ID NO: 132	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTSSNYAN SEQ ID NO: 421	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11830 H1_L1.15 SEQ ID NO: 136	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTSGHYAN SEQ ID NO: 422	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11831 H1_L1.16 SEQ ID NO: 140	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	DTNKRAP SEQ ID NO: 426	ALWYSNLWV SEQ ID NO: 430

Figure 30D

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP11832 H1_L1.17 SEQ ID NO: 144	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNNRAP SEQ ID NO: 427	ALWYSNLWV SEQ ID NO: 430
XENP11833 H1_L1.18 SEQ ID NO: 148	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAS SEQ ID NO: 428	ALWYSNLWV SEQ ID NO: 430
XENP11834 H1_L1.19 SEQ ID NO: 152	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTSNKHS SEQ ID NO: **462**	ALWYSNLWV SEQ ID NO: 430
XENP11835 H1_L1.20 SEQ ID NO: 156	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11836 H1_L1.21 SEQ ID NO: 160	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11837 H1_L1.22 SEQ ID NO: 164	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11838 H1_L1.23 SEQ ID NO: 168	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11839 H1_L1.24 SEQ ID NO: 172	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11840 H1_L1.25 SEQ ID NO: 176	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11841 H1_L1.26 SEQ ID NO: 180	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11842 H1_L1.27 SEQ ID NO: 184	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	LLWYSNLWV SEQ ID NO: 417
XENP11843 H1_L1.28 SEQ ID NO: 188	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433

Figure 30E

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP11844 H1_L1.29 SEQ ID NO: 192	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11920 H1_L3.1 SEQ ID NO: 196	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	RSSTGAVTTSNYAN SEQ ID NO: 423	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11921 H1_L5.1 SEQ ID NO: 200	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	KSSTGAVTTSNYAN SEQ ID NO: 424	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP11922 H1_L1.31 SEQ ID NO: 204	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP11923 H1_L1.32 SEQ ID NO: 208	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12107 H1.38_L1.4 SEQ ID NO: 212	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12108 H1_L1.33 SEQ ID NO: 216	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12109 H1_L1.34 SEQ ID NO: 220	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12110 H1_L1.35 SEQ ID NO: 224	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12111 H1_L1.36 SEQ ID NO: 228	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12112 H1_L1.37 SEQ ID NO: 232	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12113 H1_L1.38 SEQ ID NO: 236	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430

Figure 30F

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP12114 H1_L1.39 SEQ ID NO: 240	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12131 H1.30_L1.4 SEQ ID NO: 244	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12132 H1.31_L1.4 SEQ ID NO: 248	TYAMS SEQ ID NO: 412	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12133 H1.32_L1.4 SEQ ID NO: 252	TYAMN SEQ ID NO: 411	RIRSKANNYATYYADSVKG SEQ ID NO: 414	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12134 H1.33_L1.4 SEQ ID NO: 256	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFDY SEQ ID NO: 419	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12135 H1.34_L1.4 SEQ ID NO: 260	TYAMS SEQ ID NO: 412	RIRSKANNYATYYADSVKG SEQ ID NO: 414	HGNFGDSYVSWFDY SEQ ID NO: 419	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12136 H1.35_L1.4 SEQ ID NO: 264	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12137 H1.36_L1.4 SEQ ID NO: 268	TYAMN SEQ ID NO: 411	RIRSNGGSTYYADSVKG SEQ ID NO: 415	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12138 H1.37_L1.4 SEQ ID NO: 272	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12139 H1.37_L1.32 SEQ ID NO: 276	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNLWV SEQ ID NO: 430
XENP12149 H1_L1.40 SEQ ID NO: 280	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12213 H1.30_L1.40 SEQ ID NO: 284	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433

Figure 30G

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP12214 H1.8_L1.40 SEQ ID NO: 288	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12215 H1.39_L1.40 SEQ ID NO: 292	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12216 H1.40_L1.40 SEQ ID NO: 296	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12217 H1.30_L1.41 SEQ ID NO: 300	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12218 H1.8_L1.41 SEQ ID NO: 304	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12219 H1.39_L1.41 SEQ ID NO: 308	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12220 H1.40_L1.41 SEQ ID NO: 312	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12221 H1.30_L1.42 SEQ ID NO: 316	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12222 H1.8_L1.42 SEQ ID NO: 320	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12223 H1.39_L1.42 SEQ ID NO: 324	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12224 H1.40_L1.42 SEQ ID NO: 328	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12225 H1.30_L1.43 SEQ ID NO: 332	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433

Figure 30H

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP12226 H1.8_L1.43 SEQ ID NO: 336	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12227 H1.39_L1.43 SEQ ID NO: 340	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12228 H1.40_L1.43 SEQ ID NO: 344	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12229 H1.30_L1.44 SEQ ID NO: 348	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12230 H1.8_L1.44 SEQ ID NO: 352	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12231 H1.39_L1.44 SEQ ID NO: 356	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12232 H1.40_L1.44 SEQ ID NO: 360	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12233 H1.30_L1.45 SEQ ID NO: 364	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12234 H1.8_L1.45 SEQ ID NO: 368	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12235 H1.39_L1.45 SEQ ID NO: 372	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12236 H1.40_L1.45 SEQ ID NO: 376	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12237 H1.30_L1.46 SEQ ID NO: 380	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433

Figure 30I

Xen #	vhCDR1	vhCDR2	vhCDR3	vlCDR1	vlCDR2	vlCDR3
XENP12238 H1.8_L1.46 SEQ ID NO: 384	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12239 H1.39_L1.46 SEQ ID NO: 388	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12240 H1.40_L1.46 SEQ ID NO: 392	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12241 H1.30_L1.47 SEQ ID NO: 396	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12242 H1.8_L1.47 SEQ ID NO: 400	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12243 H1.39_L1.47 SEQ ID NO: 404	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGNSYVSWFAY SEQ ID NO: 416	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP12244 H1.40_L1.47 SEQ ID NO: 408	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
XENP ?? SEQ ID NO: 1 and 2	TYAMN SEQ ID NO: 411	RIRSKYNNYATYYADSVKG SEQ ID NO: 413	HGNFGDSYVSWFAY SEQ ID NO: 417	GSSTGAVTTSNYAN SEQ ID NO: 420	GTNKRAP SEQ ID NO: 425	ALWYSNHWV SEQ ID NO: 433
Consensus sequences	TYAMXaa1, wherein Xaa1 is N, S or H (SEQ ID NO:435)	R-I-R-S-K-Xaa1-N-Xaa2-Y-A-T-Xaa3-Y-Y-A-Xaa4-S-V-K-G, wherein Xaa1 is Y or A, Xaa2 is N or S, Xaa3 is Y or A and Xaa4 is D or A(SEQ ID NO:436)	H-G-N-F-G-Xaa1-S-Y-V-S-W-F-Xaa2-Y, wherein Xaa1 is N, D or Q and Xaa2 is A or D (SEQ ID NO:437)	Xaa1-S-S-T-G-A-V-T-Xaa2-Xaa3-Xaa4-Y-A-N, wherein Xaa1 is G, R or K, Xaa2 is T or S, Xaa3 is S or G and Xaa4 is N or H, (SEQ ID NO:438)	Xaa1-T-N-Xaa2-R-A-Xaa3, wherein Xaa1 is G or D, Xaa2 is K or N, and Xaa3 is P or S (SEQ ID NO:439)	Xaa1-L-W-Y-S-N-Xaa2-W-V, wherein Xaa1 is A or L and Xaa2 is L or H (SEQ ID NO:440)

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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	NOVEL HETERODIMERIC PROTEINS

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 14/155,344 filed on January 14, 2014.

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:

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LEGAL NAME OF INVENTOR

Inventor: Gregory MOORE Date (Optional): June 6, 2016

Signature: [signature]

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

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

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LEGAL NAME OF INVENTOR

Inventor: Matthew BERNETT Date (Optional): _____

Signature: Matthew J Bernett

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LEGAL NAME OF INVENTOR

Inventor: John DESJARLAIS Date (Optional) :

Signature:

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

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- ■ United States application or PCT international application number 14/155,344 filed on January 14, 2014.

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LEGAL NAME OF INVENTOR

Inventor: Rumana RASHID Date (Optional): June 03, 2016

Signature:

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

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Application Number:	15633629
International Application Number:	
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Title of Invention:	NOVEL HETERODIMERIC PROTEINS
First Named Inventor/Applicant Name:	John R. Desjarlais
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Filer:	Louis-Vu T. Nguyen/Leonor Rivera
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Attorney Docket Number:	067461-5161-US06
Receipt Date:	26-JUN-2017
Filing Date:	
Time Stamp:	20:04:04
Application Type:	Utility under 35 USC 111(a)

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	aia0015.pdf	275537 8af1ed1040e0c6e8c8ee8ffaa5ca94126a65690b	no	2

Warnings:

Information:					
2	Application Data Sheet	aia0014.pdf	1836198 539f7b37ac7fa5095d61274f9e787598f25b8d57	no	10
Warnings:					
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3		067461_5161US06_Specification.pdf	460286 a8bf6fb3b2647199ebaf433f5577b14a0094723f	yes	81
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	75	
	Claims		76	80	
	Abstract		81	81	
Warnings:					
Information:					
4	Drawings-only black and white line drawings	067461_5161US06_Drawings.pdf	2916578 54c26a9173bd7ba6b7be20a10564ae9df8d38522	no	104
Warnings:					
Information:					
5	Oath or Declaration filed	067461_5161US02_signed_inventor_Declarations.pdf	772777 a5fbc87d202756b5e2a40f47a17bae03466 6d0f7	no	4
Warnings:					
Information:					
6	Sequence Listing (Text File)	067461-5161-US06_ST25.txt	763802	no	-
Warnings:					
Information:					
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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.

SCORE Placeholder Sheet for IFW Content

Application Number: 15633629 Document Date: 06/26/2017

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.

- Drawings – Other than Black and White Line Drawings

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.

To access the documents in the SCORE database, refer to instructions below.

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- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

==========
Sequence Listing could not be accepted due to errors.
See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Anjum, Durreshwar
Timestamp: [year=2017; month=7; day=13; hr=8; min=29; sec=25; ms=775;]
==========

****
Reviewer Comments:
NOTE: This error report refers to the sequence listing CRF received by the USPTO on:6/26/2017

1.

E444 Invalid line termination: on line 24209 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24240 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24242 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24244 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24275 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24277 and tag: <223> - line length validation was not performed
E444 Invalid line termination: on line 24313 and tag: <223> - line length validation was not performed

For SEQ ID# 437-440 second set of the feature section "<223>" has as invalid line termination errors. Please follow the instructions below to make all necessary corrections.

All Sequence Listing file line terminator must be “ASCII Carriage Return plus ASCII Line Feed.”
“Sec. 1.824 Form and format for nucleotide and/or amino acid sequence Submissions in computer readable form.
(b) Computer readable form submissions must meet these format

requirements:
    (3) Line Terminator: ASCII Carriage Return plus ASCII Line Feed;”
The line terminators in the Sequences shown above has a pair of Non-ASCII Carriage Returns and a single ASCII Line Feed only. Please change the line terminators in this file to the correct “ASCII Carriage Return plus ASCII Line Feed.”

The recommended method to remove these errors is:

1 - open the sequence listing .txt file in Notepad. Do not use a word processing program such as Microsoft Word;
2 - highlight and delete all of the white space between the indicated locations;
3 - re-format the information using only the “enter key” and the “space bar”.

Please make all necessary changes.

To prevent line terminator errors in subsequent sequence listings, DO NOT edit the .txt file using word processing programs such as Microsoft Word. Word processing programs can introduce non-ASCII formatting into the file that while not visible, can be carried over into the .txt file and result in line terminator errors. Only edit the .txt file in programs such as Notepad or Wordpad.

2.

The error codes and descriptions shown below do not indicate non-compliance with the sequence rules, and do not require a response.

W213 Artificial or Unknown found in <213> in SEQ ID (1)
W213 Artificial or Unknown found in <213> in SEQ ID (2)
W213 Artificial or Unknown found in <213> in SEQ ID (3)
W213 Artificial or Unknown found in <213> in SEQ ID (4)
W213 Artificial or Unknown found in <213> in SEQ ID (5)
W213 Artificial or Unknown found in <213> in SEQ ID (6)
W213 Artificial or Unknown found in <213> in SEQ ID (7)
W213 Artificial or Unknown found in <213> in SEQ ID (8)
W213 Artificial or Unknown found in <213> in SEQ ID (9)
W213 Artificial or Unknown found in <213> in SEQ ID (10)

W213 Artificial or Unknown found in <213> in SEQ ID (11)
W213 Artificial or Unknown found in <213> in SEQ ID (12)
W213 Artificial or Unknown found in <213> in SEQ ID (13)
W213 Artificial or Unknown found in <213> in SEQ ID (14)
W213 Artificial or Unknown found in <213> in SEQ ID (15)
W213 Artificial or Unknown found in <213> in SEQ ID (16)
W213 Artificial or Unknown found in <213> in SEQ ID (17)
W213 Artificial or Unknown found in <213> in SEQ ID (18)
W213 Artificial or Unknown found in <213> in SEQ ID (19)
W213 Artificial or Unknown found in <213> in SEQ ID (20)
W447 n or Xaa used, for: SEQID(435) on line number 24155
W447 n or Xaa used, for: SEQID(436) on line number 24190
W447 n or Xaa used, for: SEQID(437) on line number 24225
W447 n or Xaa used, for: SEQID(438) on line number 24260
W447 n or Xaa used, for: SEQID(439) on line number 24298
W447 n or Xaa used, for: SEQID(440) on line number 24329

Note:

For any sequence listing-related questions or concerns the Applicant is invited to contact the USPTO Sequence Help Desk at 571-272-2510.

The errors listed above are exemplary and may not be a complete list of all errors in the sequence listing file.

To correct the sequence listing errors noted in this report - The recommended method for correction of errors is to access the sequence listing working file using the software program in which the listing was originally prepared, e.g., the project file in PatentIn, make any necessary corrections within that program, then generate a new sequence listing file. Use of a word processing program to correct errors directly in the original sequence listing file is strongly discouraged, since such programs often introduce unintended changes to the sequence listing, rendering the listing unacceptable. When the working file or original program is not available for correction, then use of a common or plain text-only editor, such as Notepad, to edit the original sequence listing file may suffice.

A sequence listing text file submitted via EFS-Web that complies with the requirements of 37 CFR 1.824(a) (2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), serves as both the paper

copy required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e).

****

Validated By CRFValidator v 1.0.5

Application No: 15633629 Version No: 1.0

Input Set:

Output Set:

Started: 2017-06-26 20:04:23.207
Finished: 2017-06-26 20:04:27.272
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 65 ms
Total Warnings: 468
Total Errors: 7
No. of SeqIDs Defined: 462
Actual SeqID Count: 462

Error code		Error Description
W	213	Artificial or Unknown found in <213> in SEQ ID (1)
W	213	Artificial or Unknown found in <213> in SEQ ID (2)
W	213	Artificial or Unknown found in <213> in SEQ ID (3)
W	213	Artificial or Unknown found in <213> in SEQ ID (4)
W	213	Artificial or Unknown found in <213> in SEQ ID (5)
W	213	Artificial or Unknown found in <213> in SEQ ID (6)
W	213	Artificial or Unknown found in <213> in SEQ ID (7)
W	213	Artificial or Unknown found in <213> in SEQ ID (8)
W	213	Artificial or Unknown found in <213> in SEQ ID (9)
W	213	Artificial or Unknown found in <213> in SEQ ID (10)
W	213	Artificial or Unknown found in <213> in SEQ ID (11)
W	213	Artificial or Unknown found in <213> in SEQ ID (12)
W	213	Artificial or Unknown found in <213> in SEQ ID (13)
W	213	Artificial or Unknown found in <213> in SEQ ID (14)
W	213	Artificial or Unknown found in <213> in SEQ ID (15)
W	213	Artificial or Unknown found in <213> in SEQ ID (16)
W	213	Artificial or Unknown found in <213> in SEQ ID (17)
W	213	Artificial or Unknown found in <213> in SEQ ID (18)
W	213	Artificial or Unknown found in <213> in SEQ ID (19)
W	213	Artificial or Unknown found in <213> in SEQ ID (20)

Input Set:

Output Set:

Started: 2017-06-26 20:04:23.207
Finished: 2017-06-26 20:04:27.272
Elapsed: 0 hr(s) 0 min(s) 4 sec(s) 65 ms
Total Warnings: 468
Total Errors: 7
No. of SeqIDs Defined: 462
Actual SeqID Count: 462

Error code	Error Description
	This error has occured more than 20 times, will not be displayed
W 447	n or Xaa used, for: SEQID(435) on line number 24155
W 447	n or Xaa used, for: SEQID(436) on line number 24190
E 444	Invalid line termination: on line 24209 and tag: <223> - line length validation was not performed
W 447	n or Xaa used, for: SEQID(437) on line number 24225
E 444	Invalid line termination: on line 24240 and tag: <223> - line length validation was not performed
E 444	Invalid line termination: on line 24242 and tag: <223> - line length validation was not performed
E 444	Invalid line termination: on line 24244 and tag: <223> - line length validation was not performed
W 447	n or Xaa used, for: SEQID(438) on line number 24260
E 444	Invalid line termination: on line 24275 and tag: <223> - line length validation was not performed
E 444	Invalid line termination: on line 24277 and tag: <223> - line length validation was not performed
W 447	n or Xaa used, for: SEQID(439) on line number 24298
E 444	Invalid line termination: on line 24313 and tag: <223> - line length validation was not performed
W 447	n or Xaa used, for: SEQID(440) on line number 24329

SEQUENCE LISTING

<110> Xencor, Inc.
Desjarlais, John
Moore, Gregory
Rashid, Rumana
Bernett, Matthew

<120> NOVEL HETERODIMERIC PROTEINS

<130> 067461-5161-US06

<140> US 15/633,629
<141> 2017-06-26

<150> US 14/155,344
<151> 2014-01-14

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<220>
<223> synthetic sequence

<400> 1

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1 5 10 15

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

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

Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp

```
    50                  55                  60

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

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

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120                 125
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<210> 2
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 2

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

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
        35                  40                  45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
    50                  55                  60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105
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<210> 3
<211> 257
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 3

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


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


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


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


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


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


Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110


Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125


Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
    130                 135                 140


Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160


Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175


Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190
```

```
Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala Gln Pro Glu Asp
    210                 215                 220

Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser His His His His His
                245                 250                 255

His
```

<210> 4
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 4

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

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

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

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

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

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

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110
```

```
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala Gln Pro Glu Asp
    210                 215                 220

Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu
                245
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<210> 5
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 5

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

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

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
```

```
        35                  40                  45

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

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

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

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120                 125
```

<210> 6
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 6

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

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
        35                  40                  45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
    50                  55                  60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95
```

```
Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105


<210>  7
<211>  257
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic sequence

<400>  7

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


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


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


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


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


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


Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110


Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125


Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
    130                 135                 140


Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160


Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175
```

```
Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala Gln Pro Glu Asp
    210                 215                 220

Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Ser His His His His His
                245                 250                 255

His
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<210> 8
<211> 249
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 8

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

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

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

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

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

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

```
Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160

Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
            180                 185                 190

Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu
        195                 200                 205

Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala Gln Pro Glu Asp
    210                 215                 220

Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn Leu Trp Val Phe
225                 230                 235                 240

Gly Gly Gly Thr Lys Leu Thr Val Leu
                245
```

<210> 9
<211> 125
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 9

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Thr Tyr
```

```
            20                  25                  30

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

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

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

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

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120                 125
```

<210> 10
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 10

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

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
        35                  40                  45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Val Pro Ala Arg Phe
    50                  55                  60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65                  70                  75                  80
```

```
Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105
```

<210> 11
<211> 257
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 11

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1               5                   10                  15

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

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

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

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

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

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
            100                 105                 110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val
    130                 135                 140

Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu
145                 150                 155                 160
```

```
Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
                165                 170                 175

Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly
```

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United States Patent and Trademark Office
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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
15/633,629	06/26/2017		0.00	067461-5161-US06	38	2

CONFIRMATION NO. 7776

67374
MORGAN, LEWIS & BOCKIUS LLP (SF)
ONE MARKET, SPEAR STREET TOWER, SUITE 2800
SAN FRANCISCO, CA 94105

FILING RECEIPT

OC000000092800768

Date Mailed: 07/17/2017

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)

John Desjarlais, Pasadena, CA;
Gregory Moore, Azusa, CA;
Rumana Rashid, Temple City, CA;
Matthew Bernett, Monrovia, CA;

Applicant(s)

Xencor, Inc., Monrovia, CA;

Power of Attorney: The patent practitioners associated with Customer Number 67374

Domestic Priority data as claimed by applicant

This application is a CON of 14/155,344 01/14/2014 PAT 9701759
which claims benefit of 61/780,310 03/13/2013
and claims benefit of 61/778,148 03/12/2013
and claims benefit of 61/764,954 02/14/2013
and claims benefit of 61/752,347 01/14/2013

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: 07/14/2017
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/633,629**
Projected Publication Date: To Be Determined - pending completion of Missing Parts
Non-Publication Request: No
Early Publication Request: No
Title

NOVEL HETERODIMERIC PROTEINS

Preliminary Class

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

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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

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

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

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

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

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

Application Number	Filing Date
15633629	

Applicant(s) John Desjarlais

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
2		1				
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Total Indep	2		0		0	
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Total Claims	41		0		0	

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UNITED STATES PATENT AND TRADEMARK OFFICE

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

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
15/633,629	06/26/2017	John Desjarlais	067461-5161-US06

CONFIRMATION NO. 7776

67374
MORGAN, LEWIS & BOCKIUS LLP (SF)
ONE MARKET, SPEAR STREET TOWER, SUITE 2800
SAN FRANCISCO, CA 94105

FORMALITIES LETTER

OC00000009280076 9

Date Mailed: 07/17/2017

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
- The application search fee must be submitted.
- The application examination fee must be submitted.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.
- A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing." Applicant must provide a substitute computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). Refer to attachment or PAIR document dated 07/13/2017.

For questions regarding compliance to these requirements, please contact:

- **For Rules interpretation, call (571) 272-2510**
- **For CRF submission help, call (571) 272-2510**
- **For PatentIn software program support technical assistance, call (866)217-9197.**
- **Send e-mail correspondence for PatentIn software program help to ebc@uspto.gov**
- **Patent software is available at http://www.uspto.gov/patents/resources/tools/checker/patentinrel.jsp .**

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

- Additional claim fees of $ **2460** as an undiscounted entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

SUMMARY OF FEES DUE:

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. No entity status discount is in effect. If applicant is qualified for small entity status, a written assertion of small entity status must be submitted to establish small entity status. (See 37 CFR 1.27). If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **280** basic filing fee.
- $ **400** for **37** electronically equivalent pages in excess of **100** application size fee.
- $ **140** surcharge.
- $ **600** search fee.
- $ **720** examination fee.
- $ **1680** for **21** total claims over 20.
- $ **780** for multiple dependent claim surcharge.
- $(**0**) previous unapplied payment amount.
- $ **4600** TOTAL FEE BALANCE DUE.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

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**.

/tle/

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/633,629

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))	41 minus 20 =	* 21			OR	x 80 =	1680
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 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).						400
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							780
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	4460

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($)	ADDITIONAL FEE($)		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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

DESJARLAIS et al.

Application No. **15/633,629**

Filed: **June 26, 2017**

Title: **NOVEL HETERODIMERIC PROTEINS**

Examiner: **Not yet assigned**

Art Unit: **Not yet assigned**

Confirmation No. **7776**

CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. 1.8

I hereby certify that this correspondence, including listed enclosures, is being electronically transmitted to the United States Patent and Trademark Office in accordance with 37 C.F.R. 1.6(a)(4) on February 20, 2018:

Signed: /Leonor Rivera-Huerta/
Leonor Rivera-Huerta

RESPONSE TO NOTICE TO FILE MISSING PARTS

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir or Madam:

In response to the Notice to File Missing Parts mailed July 17, 2017, Applicant submits the following amendments and remarks. This response is timely filed, with a petition for a five-month extension of time, the first business day following February 17, 2017.

Amendments to the Specification begin on page 8 of this paper.

Amendments to the Sequence Listing begin on page 9 of this paper.

Amendments to the Drawings begin on page 10 of this paper.

Remarks begin on page 5 of this paper.

Amendments to the Specification:

Applicant has amended the specification so that the 'Brief Description of the Drawings' section refers to all of the figures as properly labeled, as required under 37 CFR 1.52, 1.121(b)(3), and 1.125.

Applicant submits a substitute specification, excluding claims, with markings accompanied by a clean version (without markings). In accordance with 37 CFR 1.121(b)(3) and 1.125, the substitute specification contains no new matter.

Entry of these amendments is respectfully requested.

Amendments to the Sequence Listing:

Applicant submits a SUBSTITUTE SEQUENCE LISTING for this application. Applicant has removed the line termination errors from SEQ ID NOS: 437-440 and has also corrected the descriptions for the miscellaneous features found in those sequences.

No further changes to the drawings have been made by way of this amendment. In accordance with 37 CFR 1.121(b)(3) and 1.125(b**), the SUBSTITUTE SEQUENCE LISTING contain no new matter.**

Statement to Support Sequence Listing

This submission is accompanied by a computer readable form containing SEQ ID NOs:1-462. The sequence listing in computer readable form was prepared through the use of the software program "PatentIn, version 3.5" in accordance with 37 C.F.R. §§ 1.821 – 1.825.

According to the Legal Framework for EFS-Web (September 2008), if a sequence listing text file submitted via EFS-Web complies with the requirements of 37 C.F.R. § 1.824(a)(2)-(6) and (b), the text file will serve as both the paper copy required by 37 C.F.R. § 1.821(c) and the CRF required by 37 C.F.R. § 1.821(e). Therefore a paper copy of the referenced sequence listing is not included with this application.

Amendments to the Drawings

Pursuant to 37 CFR 1.84 and 37 CFR 1.121(d), Applicant has relabeled the drawings so that all of the different views of the drawings are labeled with a consecutive number and a capital letter. Accordingly, the brief description of the drawings refers to the Figures as properly labeled, as required under 37 CFR 1.52.

No further changes to the drawings have been made by way of this amendment. In accordance with 37 CFR 1.121(b)(3) and 1.125(b)**, the replacement drawings contain no new matter.**

CONCLUSION

Although Applicant does not believe any additional fees are required, the Commissioner is authorized to charge any additional required fees or to credit any overpayment to Morgan, Lewis & Bockius LLP Deposit Account No. 50-0310 (Attorney Docket No. 067461-5161-US06).

Please direct any calls in connection with this application to the undersigned at (415) 442-1000.

Respectfully submitted,

Dated: February 20, 2018

/Louis T. Nguyen/

Louis T. Nguyen, Reg. No. 58,729
MORGAN, LEWIS & BOCKIUS LLP
One Market, Spear Street Tower
San Francisco, CA 94105
Telephone: 415.442.1000
Facsimile: 415.442.1001

NOVEL HETERODIMERIC PROTEINS

PRIORITY

[0001] This application is a continuation of U.S. Patent Application No. 14/155,344, filed January 14, 2014 which claims priority to U.S. Provisional Patent Application Nos. 61/752,348 filed on January 14, 2013, No. 61/780,310 filed on March 13, 2013, No. 61/778,148 filed March 12, 2013, and No. 61/764,954 filed February 14, 2013, the entire contents of which are incorporated herein for all purposes by this reference.

SEQUENCE LISTING

[0002] 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 June 26, 2017, is named 067461-5161-US06_ST25.txt and is 763,759 bytes in size.

INCORPORATION OF RELATED APPLICATIONS

[0003] The following applications are incorporated by reference in their entirety, USSNs 61/302,707, 61/732,813, 61/598,686, 61/441,552, 61/311,472, 61/450,457, 61/545,498, 61/368,969, 61/391,509, 61/391,515, 61/439,263, 61/368,962 and 61/593,846 , 61/515,745, 61/752,349, 13/194,904, 13/568,028, 13/648,951, 12/875,015, as well as US Publication Nos. 2009/0163,699 and 2006/0024298;

TECHNICAL FIELD

[0004] The present invention describes novel immunoglobulin compositions that simultaneously co-engage antigens, where both of the antigens are bound monovalently. The novel immunoglobulins described preferably utilize heterodimeric Fc regions. Methods of using the novel immunoglobulin compositions, particularly for therapeutic purposes, are also described herein.

BACKGROUND OF THE INVENTION

[0005] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer and autoimmune/inflammatory disorders. Yet improvements to this class of drugs are still needed, particularly with respect to enhancing their clinical

efficacy. One avenue being explored is the engineering of additional and novel antigen binding sites into antibody-based drugs such that a single immunoglobulin molecule co-engages two different antigens. Such non-native or alternate antibody formats that engage two different antigens are often referred to as bispecifics. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific generation is the introduction of new variable regions into the antibody.

[0006] A number of alternate antibody formats have been explored for bispecific targeting (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). Initially, bispecific antibodies were made by fusing two cell lines that each produced a single monoclonal antibody (Milstein et al., 1983, Nature 305:537-540). Although the resulting hybrid hybridoma or quadroma did produce bispecific antibodies, they were only a minor population, and extensive purification was required to isolate the desired antibody. An engineering solution to this was the use of antibody fragments to make bispecifics. Because such fragments lack the complex quaternary structure of a full length antibody, variable light and heavy chains can be linked in single genetic constructs. Antibody fragments of many different forms have been generated, including diabodies, single chain diabodies, tandem scFv's, and Fab_2 bispecifics (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). While these formats can be expressed at high levels in bacteria and may have favorable penetration benefits due to their small size, they clear rapidly in vivo and can present manufacturing obstacles related to their production and stability. A principal cause of these drawbacks is that antibody fragments typically lack the constant region of the antibody with its associated functional properties, including larger size, high stability, and binding to various Fc receptors and ligands that maintain long half-life in serum (i.e., the neonatal Fc receptor FcRn) or serve as binding sites for purification (i.e., protein A and protein G).

[0007] More recent work has attempted to address the shortcomings of fragment-based bispecifics by engineering dual binding into full length antibody -like formats (Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297; USSN12/477,711; Michaelson et al., 2009, mAbs 1[2]:128-141; PCT/US2008/074693; Zuo et al., 2000, Protein Engineering 13[5]:361-367; USSN09/865,198; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu et al., 2005,

J Biol Chem 280[20]:19665-19672; PCT/US2005/025472; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). These formats overcome some of the obstacles of the antibody fragment bispecifics, principally because they contain an Fc region. One significant drawback of these formats is that, because they build new antigen binding sites on top of the homodimeric constant chains, binding to the new antigen is always bivalent.

[0008] For many antigens that are attractive as co-targets in a therapeutic bispecific format, the desired binding is monovalent rather than bivalent. For many immune receptors, cellular activation is accomplished by cross-linking of a monovalent binding interaction. The mechanism of cross-linking is typically mediated by antibody/antigen immune complexes, or via effector cell to target cell engagement. For example, the low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, upon immune complexation or cell-to-cell contact, receptors are cross-linked and clustered on the cell surface, leading to activation. For receptors responsible for mediating cellular killing, for example FcγRIIIa on natural killer (NK) cells, receptor cross-linking and cellular activation occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, expressly incorporated by reference). Similarly, on B cells the inhibitory receptor FcγRIIb downregulates B cell activation only when it engages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). As another example, CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Indeed nonspecific bivalent cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622–632; expressly incorporated by reference). Thus for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged target.

[0009] Thus while bispecifics generated from antibody fragments suffer biophysical and pharmacokinetic hurdles, a drawback of those built with full length antibody -like formats is that they engage co-target antigens multivalently in the absence of the primary target antigen, leading to nonspecific activation and potentially toxicity. The present invention solves this problem by introducing a novel bispecific format that enables the co-engagement of distinct target antigens.

SUMMARY OF THE INVENTION

[0010] Accordingly, in one aspect, the invention provides heterodimeric antibodies comprising a first heavy chain comprising a first Fc domain and a single chain Fv region that binds a first antigen. The heterodimeric antibodies also comprise a second heavy chain comprising a second Fc domain, a first variable heavy chain and a first variable light chain, wherein the first and second Fc domains are different.

[0011] In a further aspect, the pIs of the first heavy chain and the second heavy chain are at least 0.5 logs apart.

[0012] In an additional aspect, the heterodimeric antibodies of the invention further comprise one or two additional antigen binding domains that can be either or both of an scFv and a Fab.

[0013] In a further aspect, the heterodimeric antibodies of the invention comprise heterodimerization variants according to Figures 4, 5, 6, 20, 21 and 22.

[0014] In an additional aspect, the heterodimeric antibodies comprise pI variants in each monomer, with monomer one are ISO(-): I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL44 and the pI subsitutions in monomer 2 are ISO(+RR): Q196K/I199T/P217R/P228R/N276K.

[0015] In additional aspects, the present invention provides heterodimeric antibodies wherein one of the heavy chains comprises a variant selected from the group consisting of S119E, K133E, K133Q, R133E (in case of IgG2-4), R133Q (in case of IgG2-4), T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, Deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E, 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S,

370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, 439D, 349C/364E, 349K/351K, 349K/351K/394F, 349K/354C, 349K/394F, 349K/394F/401K, 349K/394Y, 349K/401K, 349K/405A, 349T/351E/411E, 349T/394F, 349T/394F/401K, 349T/394F/411E, 349T/405A, 349T/411E, 351E/364D, 351E/364D/405A, 351E/364E, 351E/366D, 351K/364H/401K, 351K/366K, 364D/370G, 364D/394F, 364E/405A, 364E/405S, 364E/411E, 364E/411E/405A, 364H/394F, 364H/401K, 364H/401K/405A, 364H/405A, 364H/405A/411E, 364Y/370R, 370E/411E, 370R/411K, 395T/397S/405A and 397S/405A.

[0016] The present invention describes methods for generating the novel compositions of the invention. The present invention describes purification methods for the immunogloublins herein, particularly methods for separating heterodimeric and homodimeric protein species. Also described are methods of testing the immunoglobulins herein, including in vitro and in vitro experiments.

[0017] The present invention provides isolated nucleic acids encoding the novel immunoglobulin compositions 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 immunoglobulin compositions.

[0018] The present invention provides compositions comprising immunoglobulin polypeptides described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[0019] The present invention contemplates therapeutic and diagnostic uses for the immunoglobulin polypeptides disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures for bottle cap

[0020] Figure 1A-1C. Illustration of the "triple F" format for bispecific immunoglobulins. Figure 1A shows a scFv-Fc format. Figure 1C depicts a more standard bispecific format, also utilizing the pI variants of the invention (and optionally and independently the other heterodimerization variants). Figure 1B shows the "triple F" format (sometimes also referred to as the "bottle-opener" configuration; (and optionally and independently the other heterodimerization variants).

[0021] Figures 2A-2E depict a variety of "higher multispecificity" embodiments of the present invention. Figure 2A shows a "triple F" configuration with another scFv attached to the Fab monomer (this one, along with Figure 2D, has a greater molecular weight differential as well). Figure 2B depicts a "triple F" with another scFv attached to the scFv monomer. Figure 2C depicts a "three scFv" format. Figure 2D depicts an additional Fab attached to the Fab monomer. Figure 2E depicts a Fab hooked to one of the scFv monomers.

[0022] Figures 3A-3E show additional varieties of "higher multispecificity" embodiments of the "triple F" format, all with one monomer comprising an scFv and all of which have molecular weight differentials which can be exploited for purification of the heterodimers). Figure 3A shows a "Fab-Fv" format with binding to two different antigens, with Figure 3B depicting the "Fab-Fv" format with binding to a single antigen (e.g. bivalent binding to antigen 1). Figures 3C and 3D depicts a "Fv-Fab" format with similar bivalent or monovalent additional antigen binding. Figure 3E depicts one monomer with a CH1-CL attached to the second scFv.

[0023] Figure 4A and 4B show novel steric variants. As will be understood by those in the art, the first column of each table represents "corresponding" monomer pairs or "sets": that is, monomer 1 has 405A and the corresponding steric variant is 394F. Again, these can be optionally and independently combined, as long as some "strandedness" is maintained.

[0024] Figure 5A-5B depicts heterodimerization variants that find particular use in the present invention.

[0025] Figure 6 depicts novel pI heterodimerization variants of particular use in the creation of heterodimeric proteins. As will be understood by those in the art, the first row of each table represents "corresponding" monomer pairs or sets. Note that the I199T and V397M variants are "neutral" (e.g. not for the purposes of altering pI of one of the monomers). Rather, they contribute to the reduction of immunogenicity by helping preserve a close epitope; e..g I199T follows pI variants N203D or Q196K, and V397M follows K392N. Thus these two variants can optionally and independently be removed from any of the sequences above.

[0026] Figure 7A-7B. Amino acid sequences for an anti-CD19 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0027] Figure 8. Amino acid sequences for the humanized anti-CD3 variable regions finding particular use in the invention (with CDRs underlined).

[0028] Figure 9A-9C. Production of XENP11874, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 9A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 9B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 9C).

[0029] Figure 10. XENP11874, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for B cell depletion.

[0030] Figure 11A-11C. The production of XENP11924, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 11A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 11B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 11C).

[0031] Figure 12. XENP11924, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the Raji tumor cell line.

[0032] Figure 13. Amino acid sequences for the anti-CD38 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0033] Figure 14A-14C. The production of XENP11925, a "triple F" bispecific with an anti-CD38 Fab and anti-CD3 scFv. In Fig. 14A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 14B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 14C).

[0034] Figure 15. XENP11925, anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the RPMI8226 tumor cell line.

[0035] Figure 16A-16B. Amino acid sequences for embodiments of the anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain for each bispecific. Amino acid substitutions made to "skew" the

formation toward desired "triple F" heterodimer over undesired dual scFv-Fc and mAb homodimers are underlined.

[0036] Figure 17. Isoelectric focusing (IEF) gel providing analysis of various "triple F" bispecifics. The desired "triple F" heterodimer has an apparent pI of 8.4 (middle band). Note the increase in heterodimer yield for XENP12152 and XENP12155.

[0037] Figure 18. Analytical ion exchange chromatography providing analysis of various "triple F" bispecifics. Yield of the desired "triple F" heterodimer is boxed. Note the increase in heterodimer yield for XENP12155.

[0038] Figure 19 shows the literature pIs of the 20 amino acids. It should be noted that the listed pIs are calculated as free amino acids; the actual pI of any side chain in the context of a protein is different, and thus this list is used to show pI trends and not absolute numbers for the purposes of the invention.

[0039] Figure 20A-20F . Data table of exemplary pI-engineered variants listing:

XenP#	the internal reference number
Name (HC)	heavy chain sequence designation
SEQ ID NO (HC)	corresponding SEQ ID NO of the heavy chain sequence
Name (LC)	light chain sequence designation
SEQ ID NO (LC)	corresponding SEQ ID NO of the light chain sequence
Calc. pI	calculated pI value for the entire antibody sequence, including heavy and light chain Fv + constant domains, with the Fv of bevacizumab and the constant domains as defined in the table
#KR	number of Lys or Arg residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta KR (vs. WT)	change in the number of Lys or Arg residues relative to IgG1 wild-type sequence of bevacizumab
#DE	number of Asp or Glu residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta DE (vs. WT)	change in the number of Asp or Glu acid residues relative to IgG1 wild-type sequence of bevacizumab
Charge state	derived from the total number of Lys and Arg minus the total number of Asp and Glu residues, assuming a pH of 7
# HC Mutations vs IgG1	number of mutations in the heavy chain constant domain as compared to IgG1
# LC Mutations vs IgG1	number of mutations in the light chain constant domain as compared to IgG1
Total # of Mutations	total number of mutations in the heavy chain and light chain constant domains as compared to IgG1

[0040] It should be noted that Figure 20A-20F has SEQ ID NO:s that are associated with the sequence listing filed in USSN 13/648,951, and are hereby expressly incorporated by reference.

[0001] Figure 21A-21C. depicts a number of pI variants that can additionally find use in the present invention, which is a list of all possible reduced pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0041] Figure 22 List of all possible increased pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0042] Figure 23A-23B is an additional list of potential heterodimerization variants for use in the present invention.

[0043] Figure 24 depicts a matrix of possible combinations of heterodimerization formats, heterodimerization variants (separated into pI variants and steric variants (which includes charge pair variants), Fc variants, FcRn variants and combinations. Legend A are suitable FcRn variants: 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E and 259I/308F/428L. That is, the Triple F format of Figure 1B can have any of these FcRn variants. For clarity, as each heavy chain is different, FcRn variants (as well as the Fc variants) can reside on one or both monomers. Legend B are suitable Fc variants: 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T. (Note, additional suitable Fc variants are found in Figure 41 of US 2006/0024298, the figure and legend of which are hereby incorporated by reference in their entirety). Legend C are suitable pI variants, and these, for brevity are imported from Figure 23A-23B, again with the understanding that there is a "strandedness" to pI variants. Legend D are suitable steric variants (including charge pair variants); again, for brevity are imported from Figure 21A-21C, again with the understanding that there is a "strandedness" to steric variants. Legend E reflects the following possible combinations, again, with each variant being independently and optionally combined from

the appropriate source Legend: 1) pI variants plus FcRn variants; 2) pI variants plus Fc variants; 3) pI variants plus FcRn variants plus Fc variants; 4) steric variants plus FcRn variants; 5) steric variants plus Fc variants; 6) steric variants plus FcRn variants plus Fc variants; 7) pI variants plus steric variants plus FcRn variants; 8) pI variants plus steric variants plus Fc variants; 9) pI variants plus steric variants plus FcRn variants plus Fc variants; and 10) pI variants plus steric variants.

[0044] Figures 25A-25 depict stability-optimized, humanized anti-CD3 variant scFvs. Substitutions are given relative to the H1_L1.4 scFv sequence. Amino acid numbering is Kabat numbering.

[0045] Figures 26A-26YY depict the amino acid sequences of stability-optimized, humanized anti-CD3 variant scFvs, variable heavy and variable light sequences. (Note also that the first sequence is the histidine tagged version for ease of purification). CDRs are underlined. It should be understood that the increased stability of the optimized variable and optimized light chains (as well as the scFv chains) can be attributed to framework regions as well as the CDRs. Thus, it should be understood that the disclosure of the entire variable region includes the disclosure of the framework regions, although they are not separately numbered. In addition, the scFv linkers are shown in grey. Each scFv linker can be replaced with a charged scFv linker as depicted in Figure 7A-7B. That is, any charged scFv linker, whether positive or negative, including those depicted in Figure 7A-7B, can be substituted for the highlighted region in Figures 2A to 2YY.

[0046] Figures 27A-27B show yields after Ni-NTA purification and melting temperatures (Tm) as determined by DSF (Differential Scanning Fluorimetry) of stability-optimized, humanized anti-CD3 variant scFvs. Similar DSF stability measurements for the I2C anti-CD3 variable region in a comparable scFv format gave a Tm of 59°C.

[0047] Figures 28A-28B show fold improvement (relative to H1_L1.4 scFv) in cell surface binding affinities (IC50) of stability-optimized, humanized anti-CD3 variant scFvs as determined in a competitive binding experiment using purified human T cells from PBMCs.

[0048] Figure 29. Figure depicts the sequence of human CD3.

[0049] Figures 30A-30I depict a collation of all the CD3 vhCDR1-3 and vlCDR1-3 sequences useful in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0050] Figures 1-76 of USSN 61/593846 and the associated legends and discussion in the specification are hereby incorporated by reference.

[0051] Figure 2 and 17 of USSN 61/778,157, inclusive of all the sequences including the optimized CD3 sequences are expressly incorporated herein by reference.

I. Overview

[0052] The present invention is directed to novel constructs to provide bispecific antibodies (or, as discussed below, trispecific or tetraspecific antibodies can also be made). An ongoing problem in antibody technologies is the desire for "bispecific" (and/or multispecific) antibodies that bind to two (or more) different antigens simultaneously, in general thus allowing the different antigens to be brought into proximity and resulting in new functionalities and new therapies. In general, these antibodies are made by including genes for each heavy and light chain into the host cells. This generally results in the formation of the desired heterodimer (A-B), as well as the two homodimers (A-A and B-B). However, a major obstacle in the formation of multispecific antibodies is the difficulty in purifying the heterodimeric antibodies away from the homodimeric antibodies and/or biasing the formation of the heterodimer over the formation of the homodimers.

[0053] The present invention is generally directed to the creation of heterodimeric proteins such as antibodies that can co-engage antigens in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers.

[0054] Thus, the present invention is directed to novel immunoglobulin compositions that co-engage at least a first and a second antigen. First and second antigens of the invention are herein referred to as antigen-1 and antigen-2 respectively. One heavy chain of the antibody contains an single chain Fv ("scFv", as defined below) and the other heavy chain is a "regular" FAb format, comprising a variable heavy chain and a light chain. This structure is sometimes referred to herein as "triple F" format (sc**F**v-**F**Ab-**F**c) or the "bottle-opener" format, due to a rough visual similarity to a bottle-opener (see Figure 1A-1C). The two chains are brought together by the use of amino acid variants in the constant regions (e.g. the Fc domain and/or the hinge region) that promote the formation of heterodimeric antibodies as is described more fully below.

[0055] There are several distinct advantages to the present "triple F" format. As is known in the art, antibody analogs relying on two scFv constructs often have stability and aggregation problems, which can be alleviated in the present invention by the addition of a "regular" heavy and light chain pairing. In addition, as opposed to formats that rely on two heavy chains and two light chains, there is no issue with the incorrect pairing of heavy and light chains (e.g. heavy 1 pairing with light 2, etc.)

[0056] There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art and described more fully below, these mechanisms can be combined to ensure high heterodimerization.

[0057] One mechanism is generally referred to in the art as "knobs and holes" ("KIH"), referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figure 4A and 4B, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization. Some of these variants are shown in Figures 16, 20, 21 and 22.

[0055] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0056] In the present invention, there are two basic mechanisms that can lead to ease of purifying heterodimeric proteins; one relies on the use of pI variants, such that each monomer has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, the "triple F" format also allows separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers, as is generally outlined below.

[0057] In the present invention that utilizes pI as a separation mechanism to create the heterodimeric Triple F format, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As is outlined more fully below, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g. a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g. glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g. loss of a charge; lysine to serine). A number of these variants are shown in Figures 6, 20 and 21.

[0058] Accordingly, in this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease it's pI (wt A-+B or wt A - -B), or by increasing one region and decreasing the other region (A+ -B- or A- B+). It should be noted that in this discussion it does not matter which monomer comprises the scFv and which the Fab.

[0059] Thus, in general, a component of the present invention are amino acid variants in the constant regions of antibodies that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein to form "pI heterodimers" (when the protein is an antibody, these are referred to as "pI antibodies") by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[0060] Furthermore, as will be appreciated by those in the art and outlined herein, heterodimers can be separated from homodimers on the basis of size. For example, as shown in Figure 1A-1B, heterodimers with two scFvs (Figure 1A) can be separated by those of the "triple F" format (figure 1B) and a bispecific mAb (Figure 1C). This can be further exploited in higher valency with additional antigen binding sites being utilized. For example, in as shown in Figure 2D, one monomer will have two Fab fragments and the other will have one scFv, resulting in a differential in size and thus molecular weight. Other examples are shown in Figures 2 and 3.

[0061] In addition, as will be appreciated by those in the art and outlined herein, the format outlined herein can be expanded to provide trispecific and tetraspecific antibodies as well. In this embodiment, some variations of which are depicted in Figures 2 and 3, it will be recognized that it is possible that some antigens are bound divalently (e.g. two antigen binding sites to a single antigen; for example, A and B could be part of a typical bivalent association and C and D can be optionally present and optionally the same or different). As will be appreciated, any combination of Fab and scFvs can be utilized to achieve the desired result and combinations.

[0062] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of the heavy chain(s), a more modular approach to designing and purifying multispecific proteins, including antibodies, is provided. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[0063] In one embodiment, the heterodimeric antibody provides for monovalent engagement of one antigen using a scFv and monovalent engagement of the other antigen using a FAb. As outlined below, this format can also be varied; in some embodiments, there is monovalent engagement of three antigens, divalent engagement of one antigen and monovalent engagement of a second antigen (e.g. A and C are to the same antigen and B is to a different antigen), etc.

[0064] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904

(incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[0065] In addition to all or part of a variant heavy constant domain, one or both of the monomers may contain one or two fusion partners, such that the heterodimers form multivalent proteins. As is generally depicted in the Figures, the fusion partners are depicted as A, B, C and D, with all combinations possible. In general, A, B, C and D are selected such that the heterodimer is at least bispecific or bivalent in its ability to interact with additional proteins. In the context of the present "triple F" format, generally A and B are an scFv and a Fv (as will be appreciated, either monomer can contain the scFv and the other the Fv/Fab) and then optionally one or two additional fusion partners.

[0066] Accordingly, the present invention provides novel constructs to produce multivalent antibodies.

[0067] Furthermore, as outlined herein, additional amino acid variants may be introduced into the bispecific antibodies of the invention, to add additional functionalities. For example, amino acid changes within the Fc region can be added (either to one monomer or both) to facilitiate increased ADCC or CDC (e.g. altered binding to Fcγ receptors); to allow or increase yield of the addition of toxins and drugs (e.g. for ADC), as well as to increase binding to FcRn and/or increase serum half-life of the resulting molecules. As is further described herein and as will be appreciated by those in the art, any and all of the variants outlined herein can be optionally and independently combined with other variants.

II. Definitions

[0068] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[0069] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding is the double variant 236R/328R.

[0070] 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. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[0071] 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.

[0072] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[0073] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0074] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position

234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[0075] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233# designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[0076] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides". The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity . Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The

identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[0077] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise 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), entirely incorporated by reference). The amino acids may either be naturally occurring or synthetic (e.g. not an amino acid that is coded for by DNA); as will be appreciated by those in the art. For example, homo-phenylalanine, citrulline, ornithine and noreleucine are considered synthetic amino acids for the purposes of the invention, and both D- and L-(R or S) configured amino acids may be utilized. The variants of the present invention may comprise modifications that include the use of synthetic amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson et al., 2004, Proc Natl Acad Sci USA 101 (2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, Science 301(5635):964-7, all entirely incorporated by reference. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[0078] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[0079] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[0080] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[0081] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[0082] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[0083] 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.

[0084] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. 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/Fc ligand complex.

[0085] By "Fc gamma receptor", "FcγR" or "FcqammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. 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 FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), 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.

[0086] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life, are discussed in Legend A of Figure 24.

[0087] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The 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 immunoglobulin" as used herein is meant an unmodified immunoglobulin 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. It should be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[0088] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as a binding moiety to a target protein, as described herein)..

[0089] 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 for antibody numbering.

[0090] By "strandedness" in the context of the monomers of the heterodimeric proteins of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities.

[0091] 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. A wide number of suitable target antigens are described below.

[0092] By "target cell" as used herein is meant a cell that expresses a target antigen.

[0093] 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.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[0094] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

Heterodimeric Proteins

[0095] The present invention is directed to the generation of multispecific, particularly bispecific binding proteins, and in particular, multispecific antibodies that have one monomer comprising an scFv and the other an Fv.

Antibody

[0096] The present invention relates to the generation of multispecific antibodies, generally therapeutic antibodies. As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. In general, the term "antibody" includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL.

[0097] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the present invention covers pI engineering of IgG1/G2 hybrids.

[0098] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[0099] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00100] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00101] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat et al., supra (1991)).

[00102] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00103] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00104] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a

polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00105] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning."

[00106] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[0100] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[0101] It should be noted that the sequences depicted herein start at the CH1 region, position 118; the variable regions are not included except as noted. For example, the first amino acid of SEQ ID NO: 2, while designated as position"1" in the sequence listing, corresponds to position 118 of the CH1 region, according to EU numbering.

[0102] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the

numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[0103] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[0104] Another region of interest for additional substitutions, outlined below, is the Fc region. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, 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, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0105] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[0106] Alternatively, the antibodies can include a variety of structures, including, but not limited to, antibody fragments,, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[0107] In one embodiment, the antibody is an antibody fragment, as long as it contains at least one constant domain which can be engineered to produce heterodimers, such as pI engineering. Other antibody fragments that can be used include fragments that contain one or

more of the CH1, CH2, CH3, hinge and CL domains of the invention that have been pI engineered. For example, Fc fusions are fusions of the Fc region (CH2 and CH3, optionally with the hinge region) fused to another protein. A number of Fc fusions are known the art and can be improved by the addition of the heterodimerization variants of the invention. In the present case, antibody fusions can be made comprising CH1; CH1, CH2 and CH3; CH2; CH3; CH2 and CH3; CH1 and CH3, any or all of which can be made optionally with the hinge region, utilizing any combination of heterodimerization variants described herein.

[0108] In some embodiments of the present invention, one monomer comprises a heavy chain comprises a scFV linked to an Fc domain, and the other monomer comprises a heavy chain comprising a Fab linked to an Fc domain, e.g. a "typical" heavy chain, and a light chain. By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

Chimeric and Humanized Antibodies

[0109] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely

incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

Multispecific Antibody Constructs

[0110] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention take on a number variety of configurations, with a preferred embodiment shown in Figure 1B as a "triple F" construct.

Heterodimeric Heavy Chain Constant Regions

[0111] Accordingly, the present invention provides heterodimeric proteins based on the use of monomers containing variant heavy chain constant regions as a first domain. By "monomer" herein is meant one half of the heterodimeric protein. It should be noted that traditional antibodies are actually tetrameric (two heavy chains and two light chains). In the context of the present invention, one pair of heavy-light chains (if applicable, e.g. if the monomer comprises an Fab) is considered a "monomer". Similarly, a heavy chain region comprising the scFv is considered a monomer. In the case where an Fv region is one fusion partner (e.g. heavy and light chain) and a non-antibody protein is another fusion partner, each "half" is considered a monomer. Essentially, each monomer comprises sufficient heavy chain constant region to allow heterodimerization engineering, whether that be all the constant region, e.g. Ch1-hinge-CH2-CH3, the Fc region (CH2-CH3), or just the CH3 domain.

[0112] The variant heavy chain constant regions can comprise all or part of the heavy chain constant region, including the full length construct, CH1-hinge-CH2-CH3, or portions thereof, including for example CH2-CH3 or CH3 alone. In addition, the heavy chain region of each monomer can be the same backbone (CH1-hinge-CH2-CH3 or CH2-CH3) or different. N- and C-terminal truncations and additions are also included within the definition; for example, some pI variants include the addition of charged amino acids to the C-terminus of the heavy chain domain.

[0113] Thus, in general, one monomer of the present "triple F" construct is a scFv region-hinge-Fc domain) and the other is (VH-CH1-hinge- CH2-CH3 plus associated light chain), with heterodimerization variants, including steric and pI variants, Fc and FcRn variants, and additional antigen binding domains (with optional linkers) included in these regions.

[0114] In addition to the heterodimerization variants (e.g. steric and pI variants) outlined herein, the heavy chain regions may also contain additional amino acid substitutions, including changes for altering FcγR and FcRn binding as discussed below.

[0115] In addition, some monomers can utilize linkers between the variant heavy chain constant region and the fusion partner. For the scFv portion of the "bottle-opener", standard linkers as are known in the art can be used. In the case where additional fusion partners are made (e.g. Figures 2and 3), traditional peptide linkers can be used, including flexible linkers of glycine and serine. In some cases, the linkers for use as components of the monomer are

different from those defined below for the ADC constructs, and are in many embodiments not cleavable linkers (such as those susceptible to proteases), although cleavable linkers may find use in some embodiments.

[0116] The heterodimerization variants include a number of different types of variants, including, but not limited to, steric variants (including charge variants) and pI variants, that can be optionally and independently combined with any other variants. In these embodiments, it is important to match "monomer A" with "monomer B"; that is, if a heterodimeric protein relies on both steric variants and pI variants, these need to be correctly matched to each monomer: e.g. the set of steric variants that work (1 set on monomer A, 1 set on monomer B) is combined with pI variant sets (1 set on monomer A, 1 set on monomer B), such that the variants on each monomer are designed to achieve the desired function.

[0117] It is important to note that the heterodimerization variants outlined herein (for example, including but not limited to those variants shown in Figures 4, 5 and 6), can be optionally and independently combined with any other variants, and on any other monomer. Thus, for example, pI variants for monomer 1 from Figure 6 can be added to other heterodimerization variants for monomer 1 in Figures 5A-5B and 6 or from monomer 2. That is, what is important for the heterodimerization is that there are "sets" of variants, one set for one monomer and one set for the other. Whether these are combined from the Figures 1 to 1 (e.g. monomer 1 listings can go together) or switched (monomer 1 pI variants with monomer 2 steric variants) is irrelevant. However, as noted herein, "strandedness" should be preserved when combinations are made as outlined above. Furthermore, for the additional Fc variants (such as for FcγR binding, FcRn binding, etc.), either monomer, or both monomers, can include any of the listed variants, independently and optionally. In some cases, both monomers have the additional variants and in some only one monomer has the additional variants, or they can be combined.

Heterodimerization Variants

[0118] The present invention provides multispecific antibody formats, on a "triple F" or "bottle opener" scaffold as depicted in Figure 1A-3E.

Steric Variants

[0119] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the heterodimeric structure than to form

homodimers with the same Fc amino acid sequences. Suitable steric variants are shown in Figures 4 and 5.

[0120] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figures 4 and 5, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[0121] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0122] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876, the figure and legend of which are incorporated expressly by reference herein.

[0123] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers.

pI (Isoelectric point) Variants for Heterodimers

[0124] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[0125] Preferred combinations of pI variants are shown in Figure 6, with additional pI variants disclosed in Figures 20, 21 and 22.

Heavy Chain Acidic pI Changes

[0126] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more positive (e.g. lower the pI), one or more of the following substitutions can be made: S119E, K133E, K133Q, T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, a deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

[0127] In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

Basic pI changes

[0128] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more negative (e.g. increase the pI), one or more of the following substitutions can be made: Q196K, P217R, P228R, N276K and H435R. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

Antibody Heterodimers Light chain variants

[0129] In the case of antibody based heterodimers, e.g. where at least one of the monomers comprises a light chain in addition to the heavy chain domain, pI variants can also be made in the light chain. Amino acid substitutions for lowering the pI of the light chain include, but are not limited to, K126E, K126Q, K145E, K145Q, N152D, S156E, K169E, S202E, K207E and adding peptide DEDE at the c-terminus of the light chain. Changes in this category based on the constant lambda light chain include one or more substitutions at R108Q, Q124E,

K126Q, N138D, K145T and Q199E. In addition, increasing the pI of the light chains can also be done.

Isotypic Variants

[0130] In addition, many embodiments of the invention rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significant affect the pI of the variant antibody. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[0131] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g. by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[0132] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

Calculating pI

[0133] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figures. Alternatively, the pI of each monomer can be compared.

pI Variants that also confer better FcRn in vivo binding

[0134] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[0135] Although still under examination, Fc regions are believed to have longer half-lives *in vivo*, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice, Dall' Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half life as wild-type Fc (Dall' Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life *in vivo* will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[0136] Recently it has been suggested that antibodies with variable regions that have lower isoelectric points may also have longer serum half-lives (Igawa et al., 2010 PEDS. 23(5): 385-392, entirely incorporated by reference). However, the mechanism of this is still poorly understood. Moreover, variable regions differ from antibody to antibody. Constant region variants with reduced pI and extended half-life would provide a more modular approach to improving the pharmacokinetic properties of antibodies, as described herein.

[0137] pI variants that find use in this embodiment, as well as their use for purification optimization, are disclosed in Figure 20A-20F.

Combination of Heterodimeric Variants

[0138] As will be appreciated by those in the art, all of the recited heterodimerization variants can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the hterodimerization formats. See Figure 24.

[0139] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

Target Antigens

[0140] The immunoglobulins of the invention may target virtually any antigens. The "triple F" format is particularly beneficial for targeting two (or more) distinct antigens. (As outlined

herein, this targeting can be any combination of monovalent and divalent binding, depending on the format). Thus the immunoglobulins herein preferably co-engage two target antigens, although in some cases, three or four antigens can be monovalently engaged. Each monomer's specificity can be selected from the lists below. While the triple F immunoglobulins described herein are particularly beneficial for targeting distinct antigens, in some cases it may be beneficial to target only one antigen. That is, each monomer may have specificity for the same antigen.

[0141] Particular suitable applications of the immunoglobulins herein are co-target pairs for which it is beneficial or critical to engage each target antigen monovalently. Such antigens may be, for example, immune receptors that are activated upon immune complexation. Cellular activation of many immune receptors occurs only by cross-linking, acheived typically by antibody/antigen immune complexes, or via effector cell to target cell engagement. For some immune receptors, for example the CD3 signaling receptor on T cells, activation only upon engagement with co-engaged target is critical, as nonspecifiic cross-linking in a clinical setting can elicit a cytokine storm and toxicity. Therapeutically, by engaging such antigens monovalently rather than multivalently, using the immunoglobulins herein, such activation occurs only in response to cross-linking only in the microenvironment of the primary target antigen. The ability to target two different antigens with different valencies is a novel and useful aspect of the present invention. Examples of target antigens for which it may be therapeutically beneficial or necessary to co-engage monovalently include but are not limited to immune activating receptors such as CD3, FcγRs, toll-like receptors (TLRs) such as TLR4 and TLR9, cytokine, chemokine, cytokine receptors, and chemokine receptors. In many embodiments, one of the antigen binding sites binds to CD3, and in some embodiments it is the scFv-containing monomer.

[0142] Virtually any antigen may be targeted by the immunoglobulins herein, including but not limited to proteins, subunits, domains, motifs, and/or epitopes belonging to the following list of target antigens, which includes both soluble factors such as cytokines and membrane-bound factors, including transmembrane receptors: 17-IA, 4-1BB, 4Dc, 6-keto-PGF1a, 8-iso-PGF2a, 8-oxo-dG, A1 Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RIIB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-1-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ,

APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bcl, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b-NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, C10, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD3, CD3E, CD4, CD5, CD6, CD7, CD8, CD10, CD11a, CD11b, CD11c, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-1, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(1-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, ENA, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxin1, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor IIa, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcR1, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3),

GDF-8 (Myostatin), GDF-9, GDF-15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alpha1, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein IIb/IIIa (GP IIb/IIIa), GM-CSF, gp130, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gp120, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gp120, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, I-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/beta1, integrin alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/beta1, integrin alpha5/beta3, integrin alpha6, integrin beta1, integrin beta2, interferon gamma, IP-10, I-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein L1, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bp1, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP-3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Muc1), MUC18, Muellerian-inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, p150, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PlGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN,

R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor-associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta RIIb, TGF-beta RIII, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL R1 Apo-2, DR4), TNFRSF10B (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcR1, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSF11B (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSF1A (TNF RI CD120a, p55-60), TNFRSF1B (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL R1 TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSF1A (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1,

VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF-1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, and receptors for hormones and growth factors. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0143] Exemplary antigens that may be targeted specifically by the immunoglobulins of the invention include but are not limited to: CD20, CD19, Her2, EGFR, EpCAM, CD3, FcγRIIIa (CD16), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRI (CD64), Toll-like receptors (TLRs) such as TLR4 and TLR9, cytokines such as IL-2, IL-5, IL-13, IL-12, IL-23, and TNFα, cytokine receptors such as IL-2R, chemokines, chemokine receptors, growth factors such as VEGF and HGF, and the like. . To form the multispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0144] Particularly preferred combinations for bispecific antibodies are an antigen-binding domain to CD3 and an antigen binding domain to CD19; an antigen-binding domain to CD3 and an antigen binding domain to CD33; an antigen-binding domain to CD3 and an antigen binding domain to CD 38. Again, in many embodiments, the CD3 binding domain is the scFv, having an exemplary sequence as depicted in the Figures and/or CD3 CDRs as outlined.

[0145] The choice of suitable target antigens and co-targets depends on the desired therapeutic application. Some targets that have proven especially amenable to antibody therapy are those with signaling functions. Other therapeutic antibodies exert their effects by blocking signaling of the receptor by inhibiting the binding between a receptor and its cognate ligand. Another mechanism of action of therapeutic antibodies is to cause receptor down regulation. Other antibodies do not work by signaling through their target antigen. The choice of co-targets will depend on the detailed biology underlying the pathology of the indication that is being treated.

[0146] Monoclonal antibody therapy has emerged as an important therapeutic modality for cancer (Weiner et al., 2010, Nature Reviews Immunology 10:317-327; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). For anti-cancer treatment it may be desirable to target one antigen (antigen-1) whose expression is restricted to the cancerous cells while co-targeting a second antigen (antigen-2) that mediates some immunulogical killing activity. For other treatments it may be beneficial to co-target two antigens, for example two angiogenic factors or two growth factors, that are each known to play some role in proliferation of the tumor. Exemplary co-targets for oncology include but are not limited to HGF and VEGF, IGF-1R and VEGF, Her2 and VEGF, CD19 and CD3, CD20 and CD3, Her2 and CD3, CD19 and FcγRIIIa, CD20 and FcγRIIIa, Her2 and FcγRIIIa. An immunoglobulin of the invention may be capable of binding VEGF and phosphatidylserine; VEGF and ErbB3; VEGF and PLGF; VEGF and ROBO4; VEGF and BSG2; VEGF and CDCP1; VEGF and ANPEP; VEGF and c-MET; HER-2 and ERB3; HER-2 and BSG2; HER-2 and CDCP1; HER-2 and ANPEP; EGFR and CD64; EGFR and BSG2; EGFR and CDCP1; EGFR and ANPEP; IGF1R and PDGFR; IGF1R and VEGF; IGF1R and CD20; CD20 and CD74; CD20 and CD30; CD20 and DR4; CD20 and VEGFR2; CD20 and CD52; CD20 and CD4; HGF and c-MET; HGF and NRP1; HGF and phosphatidylserine; ErbB3 and IGF1R; ErbB3 and IGF1,2; c-Met and Her-2; c-Met and NRP1; c-Met and IGF1R; IGF1,2 and PDGFR; IGF1,2 and CD20; IGF1,2 and IGF1R; IGF2 and EGFR; IGF2 and HER2; IGF2 and CD20; IGF2 and VEGF; IGF2 and IGF1R; IGF1 and IGF2; PDGFRa and VEGFR2; PDGFRa and PLGF; PDGFRa and VEGF; PDGFRa and c-Met; PDGFRa and EGFR; PDGFRb and VEGFR2; PDGFRb and c-Met; PDGFRb and EGFR; RON and c-Met; RON and MTSP1; RON and MSP; RON and CDCP1; VGFR1 and PLGF; VGFR1 and RON; VGFR1 and EGFR; VEGFR2 and PLGF; VEGFR2 and NRP1; VEGFR2 and RON; VEGFR2 and DLL4; VEGFR2 and EGFR; VEGFR2 and ROBO4; VEGFR2 and CD55; LPA and S1P; EPHB2 and RON; CTLA4 and VEGF; CD3 and EPCAM; CD40 and IL6; CD40 and IGF; CD40 and CD56; CD40 and CD70; CD40 and VEGFR1; CD40 and DR5; CD40 and DR4; CD40 and APRIL; CD40 and BCMA; CD40 and RANKL; CD28 and MAPG; CD80 and CD40; CD80 and CD30; CD80 and CD33; CD80 and CD74; CD80 and CD2; CD80 and CD3; CD80 and CD19; CD80 and CD4; CD80 and CD52; CD80 and VEGF; CD80 and DR5; CD80 and VEGFR2; CD22 and CD20; CD22 and CD80; CD22 and CD40; CD22 and CD23; CD22 and CD33; CD22 and CD74; CD22 and CD19; CD22 and DR5; CD22 and DR4; CD22 and VEGF; CD22 and CD52; CD30 and CD20;

CD30 and CD22; CD30 and CD23; CD30 and CD40; CD30 and VEGF; CD30 and CD74; CD30 and CD19; CD30 and DR5; CD30 and DR4; CD30 and VEGFR2; CD30 and CD52; CD30 and CD4; CD138 and RANKL; CD33 and FTL3; CD33 and VEGF; CD33 and VEGFR2; CD33 and CD44; CD33 and DR4; CD33 and DR5; DR4 and CD137; DR4 and IGF1,2; DR4 and IGF1R; DR4 and DR5; DR5 and CD40; DR5 and CD137; DR5 and CD20; DR5 and EGFR; DR5 and IGF1,2; DR5 and IGFR, DR5 and HER-2, and EGFR and DLL4. Other target combinations include one or more members of the EGF/erb-2/erb-3 family.

[0147] Other targets (one or more) involved in oncological diseases that the immunoglobulins herein may bind include, but are not limited to those selected from the group consisting of: CD52, CD20, CD19, CD3, CD4, CD8, BMP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, TNF, TNFSF10, BMP6, EGF, FGF1, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GRP, IGF1, IGF2, IL12A, IL1A, IL1B, 1L2, INHA, TGFA, TGFB1, TGFB2, TGFB3, VEGF, CDK2, FGF10, FGF18, FGF2, FGF4, FGF7, IGF1R, IL2, BCL2, CD164, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CDKN3, GNRH1, IGFBP6, IL1A, IL1B, ODZ1, PAWR, PLG, TGFB1I1, AR, BRCA1, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, E2F1, EGFR, ENO1, ERBB2, ESR1, ESR2, IGFBP3, IGFBP6, IL2, INSL4, MYC, NOX5, NR6A1, PAP, PCNA, PRKCQ, PRKD1, PRL, TP53, FGF22, FGF23, FGF9, IGFBP3, IL2, INHA, KLK6, TP53, CHGB, GNRH1, IGF1, IGF2, INHA, INSL3, INSL4, PRL, KLK6, SHBG, NR1D1, NR1H3, NR1I3, NR2F6, NR4A3, ESR1, ESR2, NR0B1, NR0B2, NR1D2, NR1H2, NR1H4, NR112, NR2C1, NR2C2, NR2E1, NR2E3, NR2F1, NR2F2, NR3C1, NR3C2, NR4A1, NR4A2, NR5A1, NR5A2, NR6 μl, PGR, RARB, FGF1, FGF2, FGF6, KLK3, KRT1, APOC1, BRCA1, CHGA, CHGB, CLU, COL1A1, COL6A1, EGF, ERBB2, ERK8, FGF1, FGF10, FGF11, FGF13, FGF14, FGF16, FGF17, FGF18, FGF2, FGF20, FGF21, FGF22, FGF23, FGF3, FGF4, FGF5, FGF6, FGF7, FGF8, FGF9, GNRH1, IGF1, IGF2, IGFBP3, IGFBP6, IL12A, IL1A, IL1B, 1L2, IL24, INHA, INSL3, INSL4, KLK10, KLK12, KLK13, KLK14, KLK15, KLK3, KLK4, KLK5, KLK6, KLK9, MMP2, MMP9, MSMB, NTN4, ODZ1, PAP, PLAU, PRL, PSAP, SERPINA3, SHBG, TGFA, TIMP3, CD44, CDH1, CDH10, CDH19, CDH20, CDH7, CDH9, CDH1, CDH10, CDH13, CDH18, CDH19, CDH20, CDH7, CDH8, CDH9, ROBO2, CD44, ILK, ITGA1, APC, CD164, COL6A1, MTSS1, PAP, TGFB1I1, AGR2, AIG1, AKAP1, AKAP2, CANT1, CAV1, CDH12, CLDN3, CLN3, CYB5, CYC1, DAB21P, DES, DNCL1, ELAC2, ENO2, ENO3, FASN, FLJ12584, FLJ25530, GAGEB1, GAGEC1,

GGT1, GSTP1, HIP1, HUMCYT2A, IL29, K6HF, KAI1, KRT2A, MIB1, PART1, PATE, PCA3, PIAS2, PIK3CG, PPID, PR1, PSCA, SLC2A2, SLC33 μl, SLC43 μl, STEAP, STEAP2, TPM1, TPM2, TRPC6, ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB 1, VEGF, VEGFC, ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB 1, ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2, CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1, CCL2, CDH5, COL1A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2, BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p161NK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), ERBB2 (Her-2), ESR1, ESR2, F3 (TF), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7 (c-Jun), MKI67 (Ki-67), NGFB (GF), NGFR, NME1 (M23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE (Tie-1), TNFRSF6 (Fas), TNFSF6 (FasL), TOP2A (topoisomerase Iia), TP53, AZGP1 (zinc-a-glycoprotein), BPAG1 (plectin), CDKN1A (p21Wap1/Cip1), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS1, ID2, ITGA6 (a6 integrin), ITGB4 (b 4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), KRTHB6 (hair-specific type II keratin), MACMARCKS, MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), S100A2, SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SCGB2A2 (mammaglobin 1), SPRR1B (Spr1), THBS1, THBS2, THBS4, and TNFAIP2 (B94), RON, c-Met, CD64, DLL4, PLGF, CTLA4, phophatidylserine, ROBO4, CD80, CD22, CD40, CD23, CD28, CD80, CD55, CD38, CD70, CD74, CD30, CD138, CD56, CD33, CD2, CD137, DR4, DR5, RANKL, VEGFR2, PDGFR, VEGFR1, MTSP1, MSP, EPHB2, EPHA1, EPHA2, EpCAM, PGE2, NKG2D, LPA, SIP, APRIL, BCMA, MAPG, FLT3, PDGFR alpha, PDGFR beta, ROR1, PSMA, PSCA, SCD1, and CD59. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0148] Monoclonal antibody therapy has become an important therapeutic modality for treating autoimmune and inflammatory disorders (Chan & Carter, 2010, Nature Reviews

Immunology 10:301-316; Reichert et al., 2005, Nature Biotechnology 23[9]:1073-1078; herein expressly incorporated by reference). Many proteins have been implicated in general autoimmune and inflammatory responses, and thus may be targeted by the immunogloublins of the invention. Autoimmune and inflammatory targets include but are not limited to C5, CCL1 (I-309), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (1-TAC/IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78/LIX), CXCL6 (GCP-2), CXCL9, IL13, IL8, CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1), IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL8, IL9, LTA, LTB, MIF, SCYE1 (endothelial Monocyte-activating cytokine), SPP1, TNF, TNFSF5, IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R, ABCF1, BCL6, C3, C4A, CEBPB, CRP, ICEBERG, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP, FADD, IRAK1, IRAK2, MYD88, NCK2, TNFAIP3, TRADD, TRAF1, TRAF2, TRAF3, TRAF4, TRAF5, TRAF6, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, CD28, CD3E, CD3G, CD3Z, CD69, CD80, CD86, CNR1, CTLA4, CYSLTR1, FCER1A, FCER2, FCGR3A, GPR44, HAVCR2, OPRD1, P2RX7, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, BLR1, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CL1, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL10, CXCL11, CXCL12, CXCL13, CXCR4, GPR2, SCYE1, SDF2, XCL1, XCL2, XCR1, AMH, AMHR2, BMPR1A, BMPR1B, BMPR2, C19orf10 (IL27w), CER1, CSF1, CSF2, CSF3, DKFZp451J0118, FGF2, GFI1, IFNA1, IFNB1, IFNG, IGF1, IL1A, IL1B, IL1R1, IL1R2, IL2, IL2RA, IL2RB, IL2RG, IL3, IL4, IL4R, IL5, IL5RA, IL6, IL6R, IL6ST, IL7, IL8, IL8RA, IL8RB, IL9, IL9R, IL10, IL10RA, IL10RB, IL11, IL12RA, IL12A, IL12B, IL12RB1, IL12RB2, IL13, IL13RA1, IL13RA2, IL15, IL15RA, IL16, IL17, IL17R, IL18, IL18R1, IL19, IL20, KITLG, LEP, LTA, LTB, LTB4R, LTB4R2, LTBR, MIF, NPPB, PDGFB, TBX21, TDGF1, TGFA, TGFB1, TGFB1I1, TGFB2, TGFB3, TGFB1, TGFBR1, TGFBR2, TGFBR3, TH1L, TNF, TNFRSF1A, TNFRSF1B, TNFRSF7, TNFRSF8, TNFRSF9, TNFRSF11A, TNFRSF21, TNFSF4, TNFSF5, TNFSF6, TNFSF11, VEGF, ZFPM2, and RNF110 (ZNF144). To form the bispecific or trispecific antibodies of the

invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0149] Exemplary co-targets for autoimmune and inflammatory disorders include but are not limited to IL-1 and TNFalpha, IL-6 and TNFalpha, IL-6 and IL-1, IgE and IL-13, IL-1 and IL-13, IL-4 and IL-13, IL-5 and IL-13, IL-9 and IL-13, CD19 and FcγRIIb, and CD79 and FcγRIIb.

[0150] Immunglobulins of the invention with specificity for the following pairs of targets to treat inflammatory disease are contemplated: TNF and IL-17A; TNF and RANKL; TNF and VEGF; TNF and SOST; TNF and DKK; TNF and alphaVbeta3; TNF and NGF; TNF and IL-23p19; TNF and IL-6; TNF and SOST; TNF and IL-6R; TNF and CD-20; IgE and IL-13; IL-13 and IL23p19; IgE and IL-4; IgE and IL-9; IgE and IL-9; IgE and IL-13; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-9; IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-23p19; IL-13 and IL-9; IL-6R and VEGF; IL-6R and IL-17A; IL-6R and RANKL; IL-17A and IL-1beta; IL-1beta and RANKL; IL-1beta and VEGF; RANKL and CD-20; IL-1alpha and IL-1beta; IL-1alpha and IL-1beta.

[0151] Pairs of targets that the immunoglobulins described herein can bind and be useful to treat asthma may be determined. In an embodiment, such targets include, but are not limited to, IL-13 and IL-1beta, since IL-1beta is also implicated in inflammatory response in asthma; IL-13 and cytokines and chemokines that are involved in inflammation, such as IL-13 and IL-9; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-25; IL-13 and TARC; IL-13 and MDC; IL-13 and MIF; IL-13 and TGF-β; IL-13 and LHR agonist; IL-13 and CL25; IL-13 and SPRR2a; IL-13 and SPRR2b; and IL-13 and ADAM8. The immunoglobulins herein may have specifity for one or more targets involved in asthma selected from the group consisting of CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FGF2, IFNA1, IFNB1, IFNG, histamine and histamine receptors, IL1A, IL1B, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12A, IL12B, IL13, IL14, IL15, IL16, IL17, IL18, IL19, KITLG, PDGFB, IL2RA, IL4R, IL5RA, IL8RA, IL8RB, IL12RB1, IL12RB2, IL13RA1, IL13RA2, IL18R1, TSLP, CCLi, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, CCL19, CCL20, CCL22, CCL24,CX3CL1, CXCL1, CXCL2, CXCL3, XCLi, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CX3CR1, GPR2, XCR1, FOS, GATA3, JAK1, JAK3, STAT6, TBX21, TGFB1, TNF, TNFSF6, YY1, CYSLTR1, FCER1A, FCER2, LTB4R, TB4R2, LTBR, and Chitinase. To form the bispecific or trispecific antibodies of the invention, antibodies to any

combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0152] Pairs of targets involved in rheumatoid arthritis (RA) may be co-targeted by the invention, including but not limited to TNF and IL-18; TNF and IL-12; TNF and IL-23; TNF and IL-1beta; TNF and MIF; TNF and IL-17; and TNF and IL-15.

[0153] Antigens that may be targeted in order to treat systemic lupus erythematosus (SLE) by the immunoglobulins herein include but are not limited to CD-20, CD-22, CD-19, CD28, CD4, CD80, HLA-DRA, IL10, IL2, IL4, TNFRSF5, TNFRSF6, TNFSF5, TNFSF6, BLR1, HDAC4, HDAC5, HDAC7A, HDAC9, ICOSL, IGBP1, MS4A1, RGSI, SLA2, CD81, IFNB1, IL10, TNFRSF5, TNFRSF7, TNFSF5, AICDA, BLNK, GALNAC4S-6ST, HDAC4, HDAC5, HDAC7A, HDAC9, IL10, IL11, IL4, INHA, INHBA, KLF6, TNFRSF7, CD28, CD38, CD69, CD80, CD83, CD86, DPP4, FCER2, IL2RA, TNFRSF8, TNFSF7, CD24, CD37, CD40, CD72, CD74, CD79A, CD79B, CR2, ILIR2, ITGA2, ITGA3, MS4A1, ST6GALI, CDIC, CHSTIO, HLA-A, HLA-DRA, and NT5E.; CTLA4, B7.1, B7.2, BlyS, BAFF, C5, IL-4, IL-6, IL-10, IFN-α, and TNF-α. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0154] The immunoglobulins herein may target antigens for the treatment of multiple sclerosis (MS), inlcuding but not limited to IL-12, TWEAK, IL-23, CXCL13, CD40, CD40L, IL-18, VEGF, VLA-4, TNF, CD45RB, CD200, IFNgamma, GM-CSF, FGF, C5, CD52, and CCR2. An embodiment includes co-engagement of anti-IL-12 and TWEAK for the treatment of MS.

[0155] One aspect of the invention pertains to immunoglobulins capable of binding one or more targets involved in sepsis, in an embodiment two targets, selected from the group consisting TNF, IL-1, MIF, IL-6, IL-8, IL-18, IL-12, IL-23, FasL, LPS, Toll-like receptors, TLR-4, tissue factor, MIP-2, ADORA2A, CASP1, CASP4, IL-10, IL-1B, NFκB1, PROC, TNFRSFIA, CSF3, CCR3, ILIRN, MIF, NFκB1, PTAFR, TLR2, TLR4, GPR44, HMOX1, midkine, IRAK1, NFκB2, SERPINA1, SERPINE1, and TREM1. To form the bispecific or trispecific antibodies of the invention, antibodies to any combination of these antigens can be

made; that is, each of these antigens can be optionally and independently included or excluded from a multispecific antibody according to the present invention.

[0156] In some cases, immunoglobulins herein may be directed against antigens for the treatment of infectious diseases.

[0157] The antibodies of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities.

[0158] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[0159] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10-4 M, at least about 10-5 M, at least about 10-6 M, at least about 10-7 M, at least about 10-8 M, at least about 10-9 M, alternatively at least about 10-10 M, at least about 10-11 M, at least about 10-12 M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[0160] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.
Modified Antibodies

[0161] In addition to the modifications outlined above, other modifications can be made. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996, Nature Biotech. 14:1239-1245, entirely

incorporated by reference). In addition, there are a variety of covalent modifications of antibodies that can be made as outlined below.

[0162] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[0163] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues may also be derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole and the like.

[0164] In addition, modifications at cysteines are particularly useful in antibody-drug conjugate (ADC) applications, further described below. In some embodiments, the constant region of the antibodies can be engineered to contain one or more cysteines that are particularly "thiol reactive", so as to allow more specific and controlled placement of the drug moiety. See for example US Patent No. 7,521,541, incorporated by reference in its entirety herein.

[0165] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

[0166] Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[0167] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin.

Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[0168] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using 125I or 131I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[0169] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N--R'), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[0170] Derivatization with bifunctional agents is useful for crosslinking antibodies to a water-insoluble support matrix or surface for use in a variety of methods, in addition to methods described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cynomolgusogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440, all entirely incorporated by reference, are employed for protein immobilization.

[0171] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[0172] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine,

arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983], entirely incorporated by reference), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0173] In addition, as will be appreciated by those in the art, labels (including fluorescent, enzymatic, magnetic, radioactive, etc. can all be added to the antibodies (as well as the other compositions of the invention).

Glycosylation

[0174] Another type of covalent modification is alterations in glycosylation. In another embodiment, the antibodies disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to the antibody, wherein said carbohydrate composition differs chemically from that of a parent antibody. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. A preferred form of engineered glycoform is afucosylation, which has been shown to be correlated to an increase in ADCC function, presumably through tighter binding to the FcγRIIIa receptor. In this context, "afucosylation" means that the majority of the antibody produced in the host cells is substantially devoid of fucose, e.g. 90-95-98% of the generated antibodies do not have appreciable fucose as a component of the carbohydrate moiety of the antibody (generally attached at N297 in the Fc region). Defined functionally, afucosylated antibodies generally exhibit at least a 50% or higher affinity to the FcγRIIIa receptor.

[0175] Engineered glycoforms may be generated by a variety of methods known in the art (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, all entirely incorporated by reference; (Potelligent® technology [Biowa, Inc., Princeton, NJ]; GlycoMAb® glycosylation engineering technology [Glycart Biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells, by regulating enzymes involved in the

glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. For example, the "sugar engineered antibody" or "SEA technology" of Seattle Genetics functions by adding modified saccharides that inhibit fucosylation during production; see for example 20090317869, hereby incorporated by reference in its entirety. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an antibody can include an engineered glycoform.

[0176] Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[0177] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0178] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[0179] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has

glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306, both entirely incorporated by reference.

[0180] Removal of carbohydrate moieties present on the starting antibody (e.g. post-translationally) may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131, both entirely incorporated by reference. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350, entirely incorporated by reference. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105, entirely incorporated by reference. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[0181] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in, for example, 2005-2006 PEG Catalog from Nektar Therapeutics (available at the Nektar website) US Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, all entirely incorporated by reference. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037A1, entirely incorporated by reference.

Additional Fc Variants for Additional Functionality

[0182] In addition to pI amino acid variants, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR receptors, altered binding to FcRn receptors, etc.

[0183] Accordingly, the proteins of the invention can include amino acid modifications, including the heterodimerization variants outlined herein, which includes the pI variants

FcγR Variants

[0184] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to Fc□RIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; 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). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L and 299T.

[0185] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

Linkers

[0186] The present invention optionally provides linkers as needed, for example in the addition of additional antigen binding sites, as depicted for example in Figure 2A-2E, where "the other end" of the molecule contains additional antigen binding components. In addition, as outlined below, linkers are optionally also used in antibody drug conjugate (ADC) systems. When used to join the components of the central mAb-Fv constructs, the linker is generally a polypeptide comprising two or more amino acid residues joined by peptide bonds and are used to link one or more of the components of the present invention. Such linker polypeptides are well known in the art (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). A variety of linkers may find use in some embodiments described herein. As will be appreciated by those in the art, there are at least three different linker types used in the present invention.

[0187] "Linker" herein is also referred to as "linker sequence", "spacer", "tethering sequence" or grammatical equivalents thereof. Homo-or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated entirely by reference). A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. The linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used. Useful linkers include glycine-serine polymers, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one, glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use as linkers.

[0188] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example Cκ or Cλ. Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example Cγ1, Cγ2, Cγ3, Cγ4, Cα1, Cα2, Cδ, Cε, and Cμ. Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

Antibody-Drug Conjugates

[0189] In some embodiments, the multispecific antibodies of the invention are conjugated with drugs to form antibody-drug conjugates (ADCs). In general, ADCs are used in oncology applications, where the use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents allows for the targeted delivery of the drug moiety to tumors, which can allow higher efficacy, lower toxicity, etc. An overview of this technology is provided in Ducry et al., Bioconjugate Chem., 21:5-13 (2010), Carter et al., Cancer J.

14(3):154 (2008) and Senter, Current Opin. Chem. Biol. 13:235-244 (2009), all of which are hereby incorporated by reference in their entirety.

[0190] Thus the invention provides multispecific antibodies conjugated to drugs. Generally, conjugation is done by covalent attachment to the antibody, as further described below, and generally relies on a linker, often a peptide linkage (which, as described below, may be designed to be sensitive to cleavage by proteases at the target site or not). In addition, as described above, linkage of the linker-drug unit (LU-D) can be done by attachment to cysteines within the antibody. As will be appreciated by those in the art, the number of drug moieties per antibody can change, depending on the conditions of the reaction, and can vary from 1:1 to 10:1 drug:antibody. As will be appreciated by those in the art, the actual number is an average.

[0191] Thus the invention provides multispecific antibodies conjugated to drugs. As described below, the drug of the ADC can be any number of agents, including but not limited to cytotoxic agents such as chemotherapeutic agents, growth inhibitory agents, toxins (for example, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (that is, a radioconjugate) are provided. In other embodiments, the invention further provides methods of using the ADCs.

[0192] Drugs for use in the present invention include cytotoxic drugs, particularly those which are used for cancer therapy. Such drugs include, in general, DNA damaging agents, anti-metabolites, natural products and their analogs. Exemplary classes of cytotoxic agents include the enzyme inhibitors such as dihydrofolate reductase inhibitors, and thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, diynenes, the podophyllotoxins, dolastatins, maytansinoids, differentiation inducers, and taxols.

[0193] Members of these classes include, for example, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosideine, actinomycin, daunorubicin, doxorubicin, mitomycin C, mitomycin A, caminomycin, aminopterin, tallysomycin, podophyllotoxin and podophyllotoxin derivatives such as etoposide or etoposide phosphate, vinblastine, vincristine, vindesine, taxanes including taxol, taxotere retinoic acid, butyric acid, N8-acetyl spermidine, camptothecin, calicheamicin, esperamicin, ene-diynes, duocarmycin A, duocarmycin SA, calicheamicin,

camptothecin, maytansinoids (including DM1), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and maytansinoids (DM4) and their analogues.

[0194] Toxins may be used as antibody-toxin conjugates and include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) J. Nat. Cancer Inst. 92(19):1573-1581; Mandler et al (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). Toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[0195] Conjugates of a multispecific antibody and one or more small molecule toxins, such as a maytansinoids, dolastatins, auristatins, a trichothecene, calicheamicin, and CC1065, and the derivatives of these toxins that have toxin activity, are contemplated.

Maytansinoids

[0196] Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. As described below, drugs may be modified by the incorporation of a functionally active group such as a thiol or amine group for conjugation to the antibody.

[0197] Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared by demethylation using Streptomyces or Actinomyces or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (--OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared by acylation using acyl chlorides) and those having modifications at other positions

[0198] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared by the reaction of maytansinol with H2S or P2S5); C-14-alkoxymethyl(demethoxy/CH2OR) (U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH2OH or CH2OAc) (U.S. Pat. No. 4,450,254) (prepared from Nocardia); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared by the

conversion of maytansinol by Streptomyces); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (isolated from Trewia nudlflora); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

[0199] Of particular use are DM1 (disclosed in US Patent No. 5,208,020, incorporated by reference) and DM4 (disclosed in US Patent No. 7,276,497, incorporated by reference). See also a number of additional maytansinoid derivatives and methods in 5,416,064, WO/01/24763, 7,303,749, 7,601,354, USSN 12/631,508, WO02/098883, 6,441,163, 7,368,565, WO02/16368 and WO04/1033272, all of which are expressly incorporated by reference in their entirety.

[0200] ADCs containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described ADCs comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

[0201] Chari et al., Cancer Research 52:127-131 (1992) describe ADCs in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3x105 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Auristatins and Dolastatins

[0202] In some embodiments, the ADC comprises a multispecific antibody conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Pat. Nos. 5,635,483; 5,780,588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001)

Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al (1998) Antimicrob. Agents Chemother. 42:2961-2965). The dolastatin or auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

[0203] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented Mar. 28, 2004 and described in United States Patent Publication No. 2005/0238648, the disclosure of which is expressly incorporated by reference in its entirety.

[0204] An exemplary auristatin embodiment is MMAE (see US Patent No. 6,884,869 expressly incorporated by reference in its entirety).

[0205] Another exemplary auristatin embodiment is MMAF (see US 2005/0238649, 5,767,237 and 6,124,431, expressly incorporated by reference in their entirety).

[0206] Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):

[0207] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schroder and K. Lubke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al (1998) Anti-Cancer Drug Design 13:243-277; Pettit, G. R., et al. Synthesis, 1996, 719-725; Pettit et al (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863; and Doronina (2003) Nat Biotechnol 21(7):778-784.

Calicheamicin

[0208] In other embodiments, the ADC comprises an antibody of the invention conjugated to one or more calicheamicin molecules. For example, Mylotarg is the first commercial ADC drug and utilizes calicheamicin $\gamma 1$ as the payload (see US Patent No. 4,970,198, incorporated by reference in its entirety). Additional calicheamicin derivatives are described in US Patent Nos. 5,264,586, 5,384,412, 5,550,246, 5,739,116, 5,773,001, 5,767,285 and 5,877,296, all

expressly incorporated by reference. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ1I, α2I, α2I, N-acetyl- γ1I, PSAG and θI1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

Duocarmycins

[0209] CC-1065 (see 4,169,888, incorporated by reference) and duocarmycins are members of a family of antitumor antibiotics utilized in ADCs. These antibiotics appear to work through sequence-selectively alkylating DNA at the N3 of adenine in the minor groove, which initiates a cascade of events that result in apoptosis.

[0210] Important members of the duocarmycins include duocarmycin A (US Patent No. 4,923,990, incorporated by reference) and duocarmycin SA (U.S. Pat. No. 5,101,038, incorporated by reference), and a large number of analogues as described in US Patent Nos. 7,517,903, 7,691,962, 5,101,038; 5,641,780; 5,187,186; 5,070,092; 5,070,092; 5,641,780; 5,101,038; 5,084,468, 5,475,092, 5,585,499, 5,846,545, WO2007/089149, WO2009/017394A1, 5,703,080, 6,989,452, 7,087,600, 7,129,261, 7,498,302, and 7,507,420, all of which are expressly incorporated by reference.

Other Cytotoxic Agents

[0211] Other antitumor agents that can be conjugated to the antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Pat. Nos. 5,053,394, 5,770,710, as well as esperamicins (U.S. Pat. No. 5,877,296).

[0212] Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin,

Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993.

[0213] The present invention further contemplates an ADC formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0214] For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu.

[0215] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as Tc99m or I123, Re186, Re188 and In111 can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate Iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[0216] For compositions comprising a plurality of antibodies, the drug loading is represented by p, the average number of drug molecules per Antibody. Drug loading may range from 1 to 20 drugs (D) per Antibody. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Antibody-Drug-Conjugates in terms of p may also be determined.

[0217] In some instances, separation, purification, and characterization of homogeneous Antibody-Drug-conjugates where p is a certain value from Antibody-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In exemplary embodiments, p is 2, 3, 4, 5, 6, 7, or 8 or a fraction thereof.

[0218] The generation of Antibody-drug conjugate compounds can be accomplished by any technique known to the skilled artisan. Briefly, the Antibody-drug conjugate compounds can

include a multispecific antibody as the Antibody unit, a drug, and optionally a linker that joins the drug and the binding agent.

[0219] A number of different reactions are available for covalent attachment of drugs and/or linkers to binding agents. This is can be accomplished by reaction of the amino acid residues of the binding agent, for example, antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. A commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule.

[0220] Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present invention.

[0221] In some embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug under appropriate conditions. In other embodiments, reactive groups are used on the drug and/or the intermediate. The product of the reaction between the drug and the intermediate, or the derivatized drug, is subsequently reacted with an multispecific antibody of the invention under appropriate conditions.

[0222] It will be understood that chemical modifications may also be made to the desired compound in order to make reactions of that compound more convenient for purposes of preparing conjugates of the invention. For example a functional group e.g. amine, hydroxyl, or sulfhydryl, may be appended to the drug at a position which has minimal or an acceptable effect on the activity or other properties of the drug

Linker Units

[0223] Typically, the antibody-drug conjugate compounds comprise a Linker unit between the drug unit and the antibody unit. In some embodiments, the linker is cleavable under intracellular or extracellular conditions, such that cleavage of the linker releases the drug unit from the antibody in the appropriate environment. For example, solid tumors that secrete

certain proteases may serve as the target of the cleavable linker; in other embodiments, it is the intracellular proteases that are utilized. In yet other embodiments, the linker unit is not cleavable and the drug is released, for example, by antibody degradation in lysosomes.

[0224] In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long or more.

[0225] Cleaving agents can include,without limitation, cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Peptidyl linkers that are cleavable by enzymes that are present in CD38-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker (SEQ ID NO: 460)). Other examples of such linkers are described, e.g., in U.S. Pat. No. 6,214,345, incorporated herein by reference in its entirety and for all purposes.

[0226] In some embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker).

[0227] In other embodiments, the cleavable linker is pH-sensitive, that is, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) may be used. (See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929).

[0228] In yet other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Pat. No. 4,880,935.)

[0229] In other embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

[0230] In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety and for all purposes).

[0231] In many embodiments, the linker is self-immolative. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. See for example, WO 2007059404A2, WO06110476A2, WO05112919A2, WO2010/062171, WO09/017394, WO07/089149, WO 07/018431, WO04/043493 and WO02/083180, which are directed to drug-cleavable substrate conjugates where the drug and cleavable substrate are optionally linked through a self-immolative linker and which are all expressly incorporated by reference.

[0232] Often the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, 15%, 10%, 5%, 3%, or no more than about 1% of the linkers, in a sample of antibody-drug conjugate compound, are cleaved when the antibody-drug conjugate compound presents in an extracellular environment (for example, in plasma).

[0233] Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the antibody-drug conjugate compound for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free drug present in the plasma.

[0234] In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent (that is, in the milieu of the linker-therapeutic agent moiety of the antibody-drug conjugate compound as described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the auristatin compound and the multispecific antibodies of the invention.

[0235] A variety of exemplary linkers that can be used with the present compositions and methods are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

Drug Loading

[0236] Drug loading is represented by p and is the average number of Drug moieties per antibody in a molecule. Drug loading ("p") may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more moieties (D) per antibody, although frequently the average number is a fraction or a decimal. Generally, drug loading of from 1 to 4 is frequently useful, and from 1 to 2 is also useful. ADCs of the invention include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy and, ELISA assay.

[0237] The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as electrophoresis.

[0238] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. $p>5$, may cause

aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the drug loading for an ADC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5. See US 2005-0238649 A1 (herein incorporated by reference in its entirety).

[0239] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0240] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachements (such as thioMab or thioFab prepared as disclosed herein and in WO2006/034488 (herein incorporated by reference in its entirety)).

[0241] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography.

[0242] In some embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Methods of Determining Cytotoxic Effect of ADCs

[0243] Methods of determining whether a Drug or Antibody-Drug conjugate exerts a cytostatic and/or cytotoxic effect on a cell are known. Generally, the cytotoxic or cytostatic activity of an Antibody Drug conjugate can be measured by: exposing mammalian cells expressing a target protein of the Antibody Drug conjugate in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays can be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the Antibody Drug conjugate.

[0244] For determining whether an Antibody Drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μCi of 3H-thymidine during the final 8 hours of the 72-hour period. The incorporation of 3H-thymidine into cells of the culture is measured in the presence and absence of the Antibody Drug conjugate.

[0245] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that an Antibody Drug conjugate is useful in the treatment of cancers.

[0246] Cell viability can be measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (see, e.g., Page et al., 1993, Intl. J. Oncology 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan et al., 1990, J. Natl. Cancer Inst. 82:1107-12).

[0247] Alternatively, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, J. Immunol. Methods 65:55-63).

[0248] Apoptosis can be quantitated by measuring, for example, DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica, 1999, no. 2, pp. 34-37 (Roche Molecular Biochemicals).

[0249] Apoptosis can also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al. eds., 1992, pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[0250] The presence of apoptotic cells can be measured in both the attached and "floating" compartments of the cultures. For example, both compartments can be collected by removing the supernatant, trypsinizing the attached cells, combining the preparations following a centrifugation wash step (e.g., 10 minutes at 2000 rpm), and detecting apoptosis (e.g., by measuring DNA fragmentation). (See, e.g., Piazza et al., 1995, Cancer Research 55:3110-16).

[0251] In vivo, the effect of a therapeutic composition of the multispecific antibody of the invention can be evaluated in a suitable animal model. For example, xenogenic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein et al., 1997, Nature Medicine 3: 402-408). Efficacy can be measured using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[0252] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate

buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

Antibody Compositions for In Vivo Administration

[0253] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0254] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to provide antibodies with other specifcities. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, growth inhibitory agent and/or small molecule antagonist. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0255] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0256] The formulations to be used for in vivo administration should be sterile, or nearly so. This is readily accomplished by filtration through sterile filtration membranes.

[0257] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0258] When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37oC, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Administrative modalities

[0259] The antibodies and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as 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. Intravenous or subcutaneous administration of the antibody is preferred.

Treatment modalities

[0260] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended

an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[0261] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[0262] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[0263] Thus for B cell tumors, the subject may experience a decrease in the so-called B symptoms, i.e., night sweats, fever, weight loss, and/or urticaria. For pre-malignant conditions, therapy with an multispecific therapeutic agent may block and/or prolong the time before development of a related malignant condition, for example, development of multiple myeloma in subjects suffering from monoclonal gammopathy of undetermined significance (MGUS).

[0264] An improvement in the disease may be characterized as a complete response. By "complete response" is intended an absence of clinically detectable disease with normalization of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF) or abnormal monoclonal protein in the case of myeloma.

[0265] Such a response may persist for at least 4 to 8 weeks, or sometimes 6 to 8 weeks, following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By "partial response" is intended at least about a 50% decrease in all measurable tumor burden (i.e., the number of malignant cells present in the subject, or the measured bulk of tumor masses or the

quantity of abnormal monoclonal protein) in the absence of new lesions, which may persist for 4 to 8 weeks, or 6 to 8 weeks.

[0266] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[0267] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[0268] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[0269] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0270] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0271] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the

particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0272] The efficient dosages and the dosage regimens for the multispecific antibodies used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[0273] An exemplary, non-limiting range for a therapeutically effective amount of an multispecific antibody used in the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, or about 3 mg/kg. In another embodiment, he antibody is administered in a dose of 1 mg/kg or more, such as a dose of from 1 to 20 mg/kg, e.g. a dose of from 5 to 20 mg/kg, e.g. a dose of 8 mg/kg.

[0274] A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician or a veterinarian could start doses of the medicament employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0275] In one embodiment, the multispecific antibody is administered by infusion in a weekly dosage of from 10 to 500 mg/kg such as of from 200 to 400 mg/kg Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours.

[0276] In one embodiment, the multispecific antibody is administered by slow continuous infusion over a long period, such as more than 24 hours, if required to reduce side effects including toxicity.

[0277] In one embodiment the multispecific antibody is administered in a weekly dosage of from 250 mg to 2000 mg, such as for example 300 mg, 500 mg, 700 mg, 1000 mg, 1500 mg or 2000 mg, for up to 8 times, such as from 4 to 6 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months. The dosage may be determined or adjusted by measuring the amount of compound of the present invention in the blood upon administration by for instance taking

out a biological sample and using anti-idiotypic antibodies which target the antigen binding region of the multispecific antibody.

[0278] In a further embodiment, the multispecific antibody is administered once weekly for 2 to 12 weeks, such as for 3 to 10 weeks, such as for 4 to 8 weeks.

[0279] In one embodiment, the multispecific antibody is administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

[0280] In one embodiment, the multispecific antibody is administered by a regimen including one infusion of an multispecific antibody followed by an infusion of an multispecific antibody conjugated to a radioisotope. The regimen may be repeated, e.g., 7 to 9 days later.

[0281] As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of an antibody in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

[0282] In some embodiments the multispecific antibody molecule thereof is used in combination with one or more additional therapeutic agents, e.g. a chemotherapeutic agent. Non-limiting examples of DNA damaging chemotherapeutic agents include topoisomerase I inhibitors (e.g., irinotecan, topotecan, camptothecin and analogs or metabolites thereof, and doxorubicin); topoisomerase II inhibitors (e.g., etoposide, teniposide, and daunorubicin); alkylating agents (e.g., melphalan, chlorambucil, busulfan, thiotepa, ifosfamide, carmustine, lomustine, semustine, streptozocin, decarbazine, methotrexate, mitomycin C, and cyclophosphamide); DNA intercalators (e.g., cisplatin, oxaliplatin, and carboplatin); DNA intercalators and free radical generators such as bleomycin; and nucleoside mimetics (e.g., 5-fluorouracil, capecitibine, gemcitabine, fludarabine, cytarabine, mercaptopurine, thioguanine, pentostatin, and hydroxyurea).

[0283] Chemotherapeutic agents that disrupt cell replication include: paclitaxel, docetaxel, and related analogs; vincristine, vinblastin, and related analogs; thalidomide, lenalidomide,

and related analogs (e.g., CC-5013 and CC-4047); protein tyrosine kinase inhibitors (e.g., imatinib mesylate and gefitinib); proteasome inhibitors (e.g., bortezomib); NF-κB inhibitors, including inhibitors of IκB kinase; antibodies which bind to proteins overexpressed in cancers and thereby downregulate cell replication (e.g., trastuzumab, rituximab, cetuximab, and bevacizumab); and other inhibitors of proteins or enzymes known to be upregulated, over-expressed or activated in cancers, the inhibition of which downregulates cell replication.

[0284] In some embodiments, the antibodies of the invention can be used prior to, concurrent with, or after treatment with Velcade® (bortezomib).

[0285] All cited references are herein expressly incorporated by reference in their entirety.

[0286] 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.

Examples

[0287] 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. For all constant region 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 Service, National Institutes of Health, Bethesda, entirely incorporated by reference). 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.
EXAMPLE 1. Prototype "triple F" bispecific antibody.

[0288] The present invention describes novel immunoglobulin compositions that co-engage a first and second antigen. One heavy chain of the antibody contains a single chain Fv ("scFv", as defined below) and the other heavy chain is a "regular" Fab format, comprising a variable heavy chain and a light chain (see Fig. 1). This structure is sometimes referred to herein as "triple F" format (scFv-Fab-Fc). The two chains are brought together by the dimeric Fc region (see Fig. 2). The Fc region can be modified by amino acid substitution to allow for efficient purification of the "triple F" heterodimer. Further, the Fc region can be

modified by amino acid substitution to promote the formation of the "triple F" heterodimer. Examples of Fc substitutions are described more fully below.

[0289] Fc substitutions can be included in the "triple F" format to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers. An example of this is in the inclusion of Fc substitutions that alter the isoelectric point (pI) of each monomer so that such that each monomer has a different pI. In this case the desired "triple F" heterodimer will have a different pI than that of the undesired dual scFv-Fc and mAb homodimers, thus facilitating isoelectric purification of the "triple F" heterodimer (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating dual scFv-Fc and mAb homodimers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns). See Fig. 3 for a list of substitutions that can be made in Fc monomer 1 and Fc monomer 2 to allow for efficient purification of the desired "triple F" heterodimer.

[0290] Fc substitutions can be included in the "triple F" format to "skew" the formation toward the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers. For example, see Fig. 4 for a list of substitutions that can be made in Fc monomer 1 and Fc monomer 2 to "skew" production toward the "triple F" heterodimer. Amino acid subsitutions listed in Fig. 3 and Fig. 4 can be combined, leading to an increased yield of "triple F" heterodimer that can be easily purified away from any contaminating dual scFv-Fc and mAb homodimers.

[0291] After optimization of an scFv domain for inclusion in the "triple F" format, an optimized scFv domain can be coupled with a variety of standard antibody heavy chains in a convenient fashion. For example, an anti-CD3 scFv for recruiting T cell cytotoxicity can be coupled with a variety of anti-tumor antigen antibody heavy chains (e.g., those binding CD5, CD20, CD30, CD40, CD33, CD38, EGFR, EpCAM, Her2, HM1.24, or other tumor antigen). Further examples of optimized scFv domains that can be conveniently coupled with standard antibody heavy chains include anti-CD16 scFv for natural killer cell cytotoxicity; anti-CD32b scFv for inhibitory activity (here the coupled antibody heavy chain would bind, e.g., CD19, CD40, CD79a, CD79b, or other immune receptors); and anti-transferrin receptor scFv, anti-insulin receptor, or anti-LRP1 for transport across the blood-brain barrier.

EXAMPLE 2. Multi-specific antibodies derived from the "triple F" format.

[0292] Multi-specific antibodies can be constructed by attaching additional scFv or Fab domains that bind a third antigen to the C-terminus of one of the "triple F" heavy chains. See Fig. 5 for examples. Alternatively, the C-terminal scFv or Fab may bind the first or second antigen, thus conferring bivalency and an increase in overall binding affinity for that antigen.

[0293] Multi-specific antibodies can also be constructed by coupling the scFv-Fc heavy chain of the "triple F" format may with rearranged antibody heavy chains as depicted in Fig. 6. Such rearranged heavy chains may include an additional Fv region that binds a third antigen or an additional Fv region that binds the first antigen or second antigen, thus conferring bivalency and an increase in overall binding affinity for that antigen.

EXAMPLE 3. Anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific.

[0294] Amino acid sequences for anti-CD19 Fab x anti-CD3 scFv "triple F" bispecifics are listed in Fig. 7. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined. Amino acid sequences for preferred humanized anti-CD3 variable regions are listed in Fig. 8 (with CDRs underlined). Some examples of expression and purification of the desired "triple F" species and its bioactivity are given below.

[0295] The production of XENP11874, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv, is outlined in Fig. 9. In Fig. 9A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 9B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 9C).

[0296] XENP11874, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11874 to potently recruit T cells for B cell depletion is shown in Fig. 10.

[0297] The production of XENP11924, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv, is outlined in Fig. 11. In Fig. 11A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 11B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 11C).

[0298] XENP11924, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11924 to potently recruit T cells for the killing of the Raji tumor cell line is shown in Fig. 12.

EXAMPLE 4. Anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific.

[0299] Amino acid sequences for anti-CD38 Fab x anti-CD3 scFv "triple F" bispecifics are listed in Fig. 13. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined. Some examples of expression and purification of the desired "triple F" species and its bioactivity are given below.

[0300] The production of XENP11925, a "triple F" bispecific with an anti-CD38 Fab and anti-CD3 scFv, is outlined in Fig. 14. In Fig. 14A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 14B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 14C).

[0301] XENP11925, anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific, was shown to have potent bioactivity. The ability of XENP11925 to potently recruit T cells for the killing of the RPMI8226 tumor cell line is shown in Fig. 15.

ABSTRACT

The invention provides heterodimeric antibodies comprising a first heavy chain comprising a first Fc domain and a single chain Fv region that binds a first antigen. The heterodimeric antibodies also comprise a second heavy chain comprising a second Fc domain, a first variable heavy chain and a first variable light chain, wherein the first and second Fc domains are different.

NOVEL HETERODIMERIC PROTEINS

PRIORITY

[0001] This application is a continuation of U.S. Patent Application No. 14/155,344, filed January 14, 2014 which claims priority to U.S. Provisional Patent Application Nos. 61/752,348 filed on January 14, 2013, No. 61/780,310 filed on March 13, 2013, No. 61/778,148 filed March 12, 2013, and No. 61/764,954 filed February 14, 2013, the entire contents of which are incorporated herein for all purposes by this reference.

SEQUENCE LISTING

[0002] 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 June 26, 2017, is named 067461-5161-US06_ST25.txt and is 763,759 bytes in size.

INCORPORATION OF RELATED APPLICATIONS

[0003] The following applications are incorporated by reference in their entirety, USSNs 61/302,707, 61/732,813, 61/598,686, 61/441,552, 61/311,472, 61/450,457, 61/545,498, 61/368,969, 61/391,509, 61/391,515, 61/439,263, 61/368,962 and 61/593,846 , 61/515,745, 61/752,349, 13/194,904, 13/568,028, 13/648,951, 12/875,015, as well as US Publication Nos. 2009/0163,699 and 2006/0024298;

TECHNICAL FIELD

[0004] The present invention describes novel immunoglobulin compositions that simultaneously co-engage antigens, where both of the antigens are bound monovalently. The novel immunoglobulins described preferably utilize heterodimeric Fc regions. Methods of using the novel immunoglobulin compositions, particularly for therapeutic purposes, are also described herein.

BACKGROUND OF THE INVENTION

[0005] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer and autoimmune/inflammatory disorders. Yet improvements to this class of drugs are still needed, particularly with respect to enhancing their clinical

efficacy. One avenue being explored is the engineering of additional and novel antigen binding sites into antibody-based drugs such that a single immunoglobulin molecule co-engages two different antigens. Such non-native or alternate antibody formats that engage two different antigens are often referred to as bispecifics. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific generation is the introduction of new variable regions into the antibody.

[0006] A number of alternate antibody formats have been explored for bispecific targeting (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). Initially, bispecific antibodies were made by fusing two cell lines that each produced a single monoclonal antibody (Milstein et al., 1983, Nature 305:537-540). Although the resulting hybrid hybridoma or quadroma did produce bispecific antibodies, they were only a minor population, and extensive purification was required to isolate the desired antibody. An engineering solution to this was the use of antibody fragments to make bispecifics. Because such fragments lack the complex quaternary structure of a full length antibody, variable light and heavy chains can be linked in single genetic constructs. Antibody fragments of many different forms have been generated, including diabodies, single chain diabodies, tandem scFv's, and Fab_2 bispecifics (Chames & Baty, 2009, mAbs 1[6]:1-9; Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; expressly incorporated herein by reference). While these formats can be expressed at high levels in bacteria and may have favorable penetration benefits due to their small size, they clear rapidly in vivo and can present manufacturing obstacles related to their production and stability. A principal cause of these drawbacks is that antibody fragments typically lack the constant region of the antibody with its associated functional properties, including larger size, high stability, and binding to various Fc receptors and ligands that maintain long half-life in serum (i.e., the neonatal Fc receptor FcRn) or serve as binding sites for purification (i.e., protein A and protein G).

[0007] More recent work has attempted to address the shortcomings of fragment-based bispecifics by engineering dual binding into full length antibody -like formats (Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297; USSN12/477,711; Michaelson et al., 2009, mAbs 1[2]:128-141; PCT/US2008/074693; Zuo et al., 2000, Protein Engineering 13[5]:361-367; USSN09/865,198; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu

et al., 2005, J Biol Chem 280[20]:19665-19672; PCT/US2005/025472; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein by reference). These formats overcome some of the obstacles of the antibody fragment bispecifics, principally because they contain an Fc region. One significant drawback of these formats is that, because they build new antigen binding sites on top of the homodimeric constant chains, binding to the new antigen is always bivalent.

[0008] For many antigens that are attractive as co-targets in a therapeutic bispecific format, the desired binding is monovalent rather than bivalent. For many immune receptors, cellular activation is accomplished by cross-linking of a monovalent binding interaction. The mechanism of cross-linking is typically mediated by antibody/antigen immune complexes, or via effector cell to target cell engagement. For example, the low affinity Fc gamma receptors (FcγRs) such as FcγRIIa, FcγRIIb, and FcγRIIIa bind monovalently to the antibody Fc region. Monovalent binding does not activate cells expressing these FcγRs; however, upon immune complexation or cell-to-cell contact, receptors are cross-linked and clustered on the cell surface, leading to activation. For receptors responsible for mediating cellular killing, for example FcγRIIIa on natural killer (NK) cells, receptor cross-linking and cellular activation occurs when the effector cell engages the target cell in a highly avid format (Bowles & Weiner, 2005, J Immunol Methods 304:88-99, expressly incorporated by reference). Similarly, on B cells the inhibitory receptor FcγRIIb downregulates B cell activation only when it engages into an immune complex with the cell surface B-cell receptor (BCR), a mechanism that is mediated by immune complexation of soluble IgG's with the same antigen that is recognized by the BCR (Heyman 2003, Immunol Lett 88[2]:157-161; Smith and Clatworthy, 2010, Nature Reviews Immunology 10:328-343; expressly incorporated by reference). As another example, CD3 activation of T-cells occurs only when its associated T-cell receptor (TCR) engages antigen-loaded MHC on antigen presenting cells in a highly avid cell-to-cell synapse (Kuhns et al., 2006, Immunity 24:133-139). Indeed nonspecific bivalent cross-linking of CD3 using an anti-CD3 antibody elicits a cytokine storm and toxicity (Perruche et al., 2009, J Immunol 183[2]:953-61; Chatenoud & Bluestone, 2007, Nature Reviews Immunology 7:622–632; expressly incorporated by reference). Thus for practical clinical use, the preferred mode of CD3 co-engagement for redirected killing of targets cells is monovalent binding that results in activation only upon engagement with the co-engaged target.

[0009] Thus while bispecifics generated from antibody fragments suffer biophysical and pharmacokinetic hurdles, a drawback of those built with full length antibody -like formats is that they engage co-target antigens multivalently in the absence of the primary target antigen, leading to nonspecific activation and potentially toxicity. The present invention solves this problem by introducing a novel bispecific format that enables the co-engagement of distinct target antigens.

SUMMARY OF THE INVENTION

[0010] Accordingly, in one aspect, the invention provides heterodimeric antibodies comprising a first heavy chain comprising a first Fc domain and a single chain Fv region that binds a first antigen. The heterodimeric antibodies also comprise a second heavy chain comprising a second Fc domain, a first variable heavy chain and a first variable light chain, wherein the first and second Fc domains are different.

[0011] In a further aspect, the pIs of the first heavy chain and the second heavy chain are at least 0.5 logs apart.

[0012] In an additional aspect, the heterodimeric antibodies of the invention further comprise one or two additional antigen binding domains that can be either or both of an scFv and a Fab.

[0013] In a further aspect, the heterodimeric antibodies of the invention comprise heterodimerization variants according to Figures 4, 5, 6, 20, 21 and 22.

[0014] In an additional aspect, the heterodimeric antibodies comprise pI variants in each monomer, with monomer one are ISO(-): I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/DEL44 and the pI subsitutions in monomer 2 are ISO(+RR): Q196K/I199T/P217R/P228R/N276K.

[0015] In additional aspects, the present invention provides heterodimeric antibodies wherein one of the heavy chains comprises a variant selected from the group consisting of S119E, K133E, K133Q, R133E (in case of IgG2-4), R133Q (in case of IgG2-4), T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, Deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E, 349A, 349C, 349E, 349I, 349K, 349S, 349T, 349W, 351E, 351K, 354C, 356K, 357K, 364C, 364D, 364E, 364F, 364G, 364H, 364R, 364T, 364Y, 366D, 366K, 366S, 366W, 366Y, 368A, 368E, 368K, 368S, 370C, 370D, 370E, 370G, 370R, 370S,

370V, 392D, 392E, 394F, 394S, 394W, 394Y, 395T, 395V, 396T, 397E, 397S, 397T, 399K, 401K, 405A, 405S, 407T, 407V, 409D, 409E, 411D, 411E, 411K, 439D, 349C/364E, 349K/351K, 349K/351K/394F, 349K/354C, 349K/394F, 349K/394F/401K, 349K/394Y, 349K/401K, 349K/405A, 349T/351E/411E, 349T/394F, 349T/394F/401K, 349T/394F/411E, 349T/405A, 349T/411E, 351E/364D, 351E/364D/405A, 351E/364E, 351E/366D, 351K/364H/401K, 351K/366K, 364D/370G, 364D/394F, 364E/405A, 364E/405S, 364E/411E, 364E/411E/405A, 364H/394F, 364H/401K, 364H/401K/405A, 364H/405A, 364H/405A/411E, 364Y/370R, 370E/411E, 370R/411K, 395T/397S/405A and 397S/405A.

[0016] The present invention describes methods for generating the novel compositions of the invention. The present invention describes purification methods for the immunogloublins herein, particularly methods for separating heterodimeric and homodimeric protein species. Also described are methods of testing the immunoglobulins herein, including in vitro and in vitro experiments.

[0017] The present invention provides isolated nucleic acids encoding the novel immunoglobulin compositions 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 immunoglobulin compositions.

[0018] The present invention provides compositions comprising immunoglobulin polypeptides described herein, and a physiologically or pharmaceutically acceptable carrier or diluent.

[0019] The present invention contemplates therapeutic and diagnostic uses for the immunoglobulin polypeptides disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures for bottle cap

[0020] Figure 1A-1C. Illustration of the "triple F" format for bispecific immunoglobulins. Figure 1A shows a scFv-Fc format. Figure 1C depicts a more standard bispecific format, also utilizing the pI variants of the invention (and optionally and independently the other heterodimerization variants). Figure 1B shows the "triple F" format (sometimes also referred to as the "bottle-opener" configuration; (and optionally and independently the other heterodimerization variants).

[0021] Figures 2A-2E depict a variety of "higher multispecificity" embodiments of the present invention. Figure 2A shows a "triple F" configuration with another scFv attached to the Fab monomer (this one, along with Figure 2D, has a greater molecular weight differential as well). Figure 2B depicts a "triple F" with another scFv attached to the scFv monomer. Figure 2C depicts a "three scFv" format. Figure 2D depicts an additional Fab attached to the Fab monomer. Figure 2E depicts a Fab hooked to one of the scFv monomers.

[0022] Figures 3A-3E show additional varieties of "higher multispecificity" embodiments of the "triple F" format, all with one monomer comprising an scFv and all of which have molecular weight differentials which can be exploited for purification of the heterodimers). Figure 3A shows a "Fab-Fv" format with binding to two different antigens, with Figure 3B depicting the "Fab-Fv" format with binding to a single antigen (e.g. bivalent binding to antigen 1). Figures 3C and 3D depicts a "Fv-Fab" format with similar bivalent or monovalent additional antigen binding. Figure 3E depicts one monomer with a CH1-CL attached to the second scFv.

[0023] Figure 4A and 4B show novel steric variants. As will be understood by those in the art, the first column of each table represents "corresponding" monomer pairs or "sets": that is, monomer 1 has 405A and the corresponding steric variant is 394F. Again, these can be optionally and independently combined, as long as some "strandedness" is maintained.

[0024] Figure 5A-5B depicts heterodimerization variants that find particular use in the present invention.

[0025] Figure 6 depicts novel pI heterodimerization variants of particular use in the creation of heterodimeric proteins. As will be understood by those in the art, the first row of each table represents "corresponding" monomer pairs or sets. Note that the I199T and V397M variants are "neutral" (e.g. not for the purposes of altering pI of one of the monomers). Rather, they contribute to the reduction of immunogenicity by helping preserve a close epitope; e..g I199T follows pI variants N203D or Q196K, and V397M follows K392N. Thus these two variants can optionally and independently be removed from any of the sequences above.

[0026] Figure 7A-7B. Amino acid sequences for an anti-CD19 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0027] Figure 8. Amino acid sequences for the humanized anti-CD3 variable regions finding particular use in the invention (with CDRs underlined).

[0028] Figure [8]9A-9C. Production of XENP11874, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 9A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 9B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 9C).

[0029] Figure 10. XENP11874, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for B cell depletion.

[0030] Figure [11]11A-11C. The production of XENP11924, a "triple F" bispecific with an anti-CD19 Fab and anti-CD3 scFv. In Fig. 11A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 11B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 11C).

[0031] Figure 12. XENP11924, anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the Raji tumor cell line.

[0032] Figure 13. Amino acid sequences for the anti-CD38 Fab x anti-CD3 scFv "triple F" embodiment, showing the HC-Fab sequence, the HC-scFv sequence, and the light chain. Amino acid substitutions made to allow for efficient purification of the desired "triple F" heterodimer over the undesired dual scFv-Fc and mAb homodimers are underlined.

[0033] Figure [14]14A-14C. The production of XENP11925, a "triple F" bispecific with an anti-CD38 Fab and anti-CD3 scFv. In Fig. 14A, the ion exchange purification of the desired "triple F" heterodimer from the undesired dual scFv-Fc and mAb homodimers is shown. The purity of the "triple F" fraction was checked by IEF gel, shown in Fig. 14B. Finally, SEC was used to confirm the homogenous size of the "triple F" product (see Fig. 14C).

[0034] Figure 15. XENP11925, anti-CD38 Fab x anti-CD3 scFv "triple F" bispecific, potently recruits T cells for the killing of the RPMI8226 tumor cell line.

[0035] Figure 16A-16B. Amino acid sequences for embodiments of the anti-CD19 Fab x anti-CD3 scFv "triple F" bispecific, showing the HC-Fab sequence, the HC-scFv sequence,

and the light chain for each bispecific. Amino acid substitutions made to "skew" the formation toward desired "triple F" heterodimer over undesired dual scFv-Fc and mAb homodimers are underlined.

[0036] Figure 17. Isoelectric focusing (IEF) gel providing analysis of various "triple F" bispecifics. The desired "triple F" heterodimer has an apparent pI of 8.4 (middle band). Note the increase in heterodimer yield for XENP12152 and XENP12155.

[0037] Figure 18. Analytical ion exchange chromatography providing analysis of various "triple F" bispecifics. Yield of the desired "triple F" heterodimer is boxed. Note the increase in heterodimer yield for XENP12155.

[0038] Figure 19 shows the literature pIs of the 20 amino acids. It should be noted that the listed pIs are calculated as free amino acids; the actual pI of any side chain in the context of a protein is different, and thus this list is used to show pI trends and not absolute numbers for the purposes of the invention.

[0039] Figure 20A-20F . Data table of exemplary pI-engineered variants listing:

XenP#	the internal reference number
Name (HC)	heavy chain sequence designation
SEQ ID NO (HC)	corresponding SEQ ID NO of the heavy chain sequence
Name (LC)	light chain sequence designation
SEQ ID NO (LC)	corresponding SEQ ID NO of the light chain sequence
Calc. pI	calculated pI value for the entire antibody sequence, including heavy and light chain Fv + constant domains, with the Fv of bevacizumab and the constant domains as defined in the table
#KR	number of Lys or Arg residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta KR (vs. WT)	change in the number of Lys or Arg residues relative to IgG1 wild-type sequence of bevacizumab
#DE	number of Asp or Glu residues in IgG1 with the Fv of bevacizumab and the constant domains as defined in the table
Delta DE (vs. WT)	change in the number of Asp or Glu acid residues relative to IgG1 wild-type sequence of bevacizumab
Charge state	derived from the total number of Lys and Arg minus the total number of Asp and Glu residues, assuming a pH of 7
# HC Mutations vs IgG1	number of mutations in the heavy chain constant domain as compared to IgG1
# LC Mutations vs IgG1	number of mutations in the light chain constant domain as compared to IgG1
Total # of Mutations	total number of mutations in the heavy chain and light chain constant domains as compared to IgG1

[0040] It should be noted that Figure 20A-20F has SEQ ID NO:s that are associated with the sequence listing filed in USSN 13/648,951, and are hereby expressly incorporated by reference.

[0001] Figure 21A-21C. depicts a number of pI variants that can additionally find use in the present invention, which is a list of all possible reduced pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0041] Figure 22 List of all possible increased pI variants created from isotypic substitutions of IgG1-4. Shown are the pI values for the three expected species as well as the average delta pI between the heterodimer and the two homodimer species present when the variant heavy chain is transfected with IgG1-WT heavy chain.

[0042] Figure 23A-23B is an additional list of potential heterodimerization variants for use in the present invention.

[0043] Figure 24 depicts a matrix of possible combinations of heterodimerization formats, heterodimerization variants (separated into pI variants and steric variants (which includes charge pair variants), Fc variants, FcRn variants and combinations. Legend A are suitable FcRn variants: 434A, 434S, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L, 252Y, 252Y/254T/256E and 259I/308F/428L. That is, the Triple F format of Figure 1B can have any of these FcRn variants. For clarity, as each heavy chain is different, FcRn variants (as well as the Fc variants) can reside on one or both monomers. Legend B are suitable Fc variants: 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 236R, 328R, 236R/328R, 236N/267E, 243L, 298A and 299T. (Note, additional suitable Fc variants are found in Figure 41 of US 2006/0024298, the figure and legend of which are hereby incorporated by reference in their entirety). Legend C are suitable pI variants, and these, for brevity are imported from Figure 23A-23B, again with the understanding that there is a "strandedness" to pI variants. Legend D are suitable steric variants (including charge pair variants); again, for brevity are imported from Figure 21A-21C, again with the understanding that there is a "strandedness" to steric variants. Legend E reflects the following possible combinations, again, with each variant being independently and optionally combined from the appropriate source Legend: 1) pI variants plus FcRn variants; 2) pI variants plus Fc

variants; 3) pI variants plus FcRn variants plus Fc variants; 4) steric variants plus FcRn variants; 5) steric variants plus Fc variants; 6) steric variants plus FcRn variants plus Fc variants; 7) pI variants plus steric variants plus FcRn variants; 8) pI variants plus steric variants plus Fc variants; 9) pI variants plus steric variants plus FcRn variants plus Fc variants; and 10) pI variants plus steric variants.

[0044] Figures 25A[-]-25[B and 25C] depict stability-optimized, humanized anti-CD3 variant scFvs. Substitutions are given relative to the H1_L1.4 scFv sequence. Amino acid numbering is Kabat numbering.

[0045] Figures 26A[-to-]-26YY depict the amino acid sequences of stability-optimized, humanized anti-CD3 variant scFvs, variable heavy and variable light sequences. (Note also that the first sequence is the histidine tagged version for ease of purification). CDRs are underlined. It should be understood that the increased stability of the optimized variable and optimized light chains (as well as the scFv chains) can be attributed to framework regions as well as the CDRs. Thus, it should be understood that the disclosure of the entire variable region includes the disclosure of the framework regions, although they are not separately numbered. In addition, the scFv linkers are shown in grey. Each scFv linker can be replaced with a charged scFv linker as depicted in Figure 7A-7B. That is, any charged scFv linker, whether positive or negative, including those depicted in Figure 7A-7B, can be substituted for the highlighted region in Figures 2A to 2YY.

[0046] Figures 27A[-and-]-27B show yields after Ni-NTA purification and melting temperatures (Tm) as determined by DSF (Differential Scanning Fluorimetry) of stability-optimized, humanized anti-CD3 variant scFvs. Similar DSF stability measurements for the I2C anti-CD3 variable region in a comparable scFv format gave a Tm of 59°C.

[0047] Figures 28A[-and-]-28B show fold improvement (relative to H1_L1.4 scFv) in cell surface binding affinities (IC50) of stability-optimized, humanized anti-CD3 variant scFvs as determined in a competitive binding experiment using purified human T cells from PBMCs.

[0048] Figure 29. Figure depicts the sequence of human CD3.

[0049] Figures 30A[-to-]-30I depict a collation of all the CD3 vhCDR1-3 and vlCDR1-3 sequences useful in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0050] Figures 1-76 of USSN 61/593846 and the associated legends and discussion in the specification are hereby incorporated by reference.

[0051] Figure 2 and 17 of USSN 61/778,157, inclusive of all the sequences including the optimized CD3 sequences are expressly incorporated herein by reference.

I. Overview

[0052] The present invention is directed to novel constructs to provide bispecific antibodies (or, as discussed below, trispecific or tetraspecific antibodies can also be made). An ongoing problem in antibody technologies is the desire for "bispecific" (and/or multispecific) antibodies that bind to two (or more) different antigens simultaneously, in general thus allowing the different antigens to be brought into proximity and resulting in new functionalities and new therapies. In general, these antibodies are made by including genes for each heavy and light chain into the host cells. This generally results in the formation of the desired heterodimer (A-B), as well as the two homodimers (A-A and B-B). However, a major obstacle in the formation of multispecific antibodies is the difficulty in purifying the heterodimeric antibodies away from the homodimeric antibodies and/or biasing the formation of the heterodimer over the formation of the homodimers.

[0053] The present invention is generally directed to the creation of heterodimeric proteins such as antibodies that can co-engage antigens in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers.

[0054] Thus, the present invention is directed to novel immunoglobulin compositions that co-engage at least a first and a second antigen. First and second antigens of the invention are herein referred to as antigen-1 and antigen-2 respectively. One heavy chain of the antibody contains an single chain Fv ("scFv", as defined below) and the other heavy chain is a "regular" FAb format, comprising a variable heavy chain and a light chain. This structure is sometimes referred to herein as "triple F" format (sc**F**v-**F**Ab-**F**c) or the "bottle-opener" format, due to a rough visual similarity to a bottle-opener (see Figure 1A-1C). The two chains are brought together by the use of amino acid variants in the constant regions (e.g. the Fc domain and/or the hinge region) that promote the formation of heterodimeric antibodies as is described more fully below.

[0055] There are several distinct advantages to the present "triple F" format. As is known in the art, antibody analogs relying on two scFv constructs often have stability and aggregation problems, which can be alleviated in the present invention by the addition of a "regular" heavy and light chain pairing. In addition, as opposed to formats that rely on two heavy chains and two light chains, there is no issue with the incorrect pairing of heavy and light chains (e.g. heavy 1 pairing with light 2, etc.)

[0056] There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art and described more fully below, these mechanisms can be combined to ensure high heterodimerization.

[0057] One mechanism is generally referred to in the art as "knobs and holes" ("KIH"), referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figure 4A and 4B, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization. Some of these variants are shown in Figures 16, 20, 21 and 22.

[0055] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0056] In the present invention, there are two basic mechanisms that can lead to ease of purifying heterodimeric proteins; one relies on the use of pI variants, such that each monomer has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, the "triple F" format also allows separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers, as is generally outlined below.

[0057] In the present invention that utilizes pI as a separation mechanism to create the heterodimeric Triple F format, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As is outlined more fully below, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g. a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g. glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g. loss of a charge; lysine to serine). A number of these variants are shown in Figures 6, 20 and 21.

[0058] Accordingly, in this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease it's pI (wt A-+B or wt A - -B), or by increasing one region and decreasing the other region (A+ -B- or A- B+). It should be noted that in this discussion it does not matter which monomer comprises the scFv and which the Fab.

[0059] Thus, in general, a component of the present invention are amino acid variants in the constant regions of antibodies that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein to form "pI heterodimers" (when the protein is an antibody, these are referred to as "pI antibodies") by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be

accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[0060] Furthermore, as will be appreciated by those in the art and outlined herein, heterodimers can be separated from homodimers on the basis of size. For example, as shown in Figure 1A-1B, heterodimers with two scFvs (Figure 1A) can be separated by those of the "triple F" format (figure 1B) and a bispecific mAb (Figure 1C). This can be further exploited in higher valency with additional antigen binding sites being utilized. For example, in as shown in Figure 2D, one monomer will have two Fab fragments and the other will have one scFv, resulting in a differential in size and thus molecular weight. Other examples are shown in Figures 2 and 3.

[0061] In addition, as will be appreciated by those in the art and outlined herein, the format outlined herein can be expanded to provide trispecific and tetraspecific antibodies as well. In this embodiment, some variations of which are depicted in Figures 2 and 3, it will be recognized that it is possible that some antigens are bound divalently (e.g. two antigen binding sites to a single antigen; for example, A and B could be part of a typical bivalent association and C and D can be optionally present and optionally the same or different). As will be appreciated, any combination of Fab and scFvs can be utilized to achieve the desired result and combinations.

[0062] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of the heavy chain(s), a more modular approach to designing and purifying multispecific proteins, including antibodies, is provided. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[0063] In one embodiment, the heterodimeric antibody provides for monovalent engagement of one antigen using a scFv and monovalent engagement of the other antigen using a FAb. As outlined below, this format can also be varied; in some embodiments, there is monovalent engagement of three antigens, divalent engagement of one antigen and monovalent engagement of a second antigen (e.g. A and C are to the same antigen and B is to a different antigen), etc.

[0064] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904 (incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[0065] In addition to all or part of a variant heavy constant domain, one or both of the monomers may contain one or two fusion partners, such that the heterodimers form multivalent proteins. As is generally depicted in the Figures, the fusion partners are depicted as A, B, C and D, with all combinations possible. In general, A, B, C and D are selected such that the heterodimer is at least bispecific or bivalent in its ability to interact with additional proteins. In the context of the present "triple F" format, generally A and B are an scFv and a Fv (as will be appreciated, either monomer can contain the scFv and the other the Fv/Fab) and then optionally one or two additional fusion partners.

[0066] Accordingly, the present invention provides novel constructs to produce multivalent antibodies.

[0067] Furthermore, as outlined herein, additional amino acid variants may be introduced into the bispecific antibodies of the invention, to add additional functionalities. For example, amino acid changes within the Fc region can be added (either to one monomer or both) to facilitiate increased ADCC or CDC (e.g. altered binding to Fcγ receptors); to allow or increase yield of the addition of toxins and drugs (e.g. for ADC), as well as to increase binding to FcRn and/or increase serum half-life of the resulting molecules. As is further described herein and as will be appreciated by those in the art, any and all of the variants outlined herein can be optionally and independently combined with other variants.

II. Definitions

[0068] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[0069] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being

below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding is the double variant 236R/328R.

[0070] 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. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[0071] 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.

[0072] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[0073] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0074] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example,

-233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[0075] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233# designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[0076] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides". The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity . Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc

variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[0077] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise 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), entirely incorporated by reference). The amino acids may either be naturally occurring or synthetic (e.g. not an amino acid that is coded for by DNA); as will be appreciated by those in the art. For example, homo-phenylalanine, citrulline, ornithine and noreleucine are considered synthetic amino acids for the purposes of the invention, and both D- and L-(R or S) configured amino acids may be utilized. The variants of the present invention may comprise modifications that include the use of synthetic amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson et al., 2004, Proc Natl Acad Sci USA 101 (2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, Science 301(5635):964-7, all entirely incorporated by reference. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation,

cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[0078] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[0079] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

[0080] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[0081] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[0082] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[0083] 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.

[0084] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. 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/Fc ligand complex.

[0085] By "Fc gamma receptor", "FcγR" or "FcqammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. 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 FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), 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.

[0086] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life, are discussed in Legend A of Figure 24.

[0087] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The 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 immunoglobulin" as used herein is meant an unmodified immunoglobulin 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. It should be

noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[0088] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as a binding moiety to a target protein, as described herein)..

[0089] 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 for antibody numbering.

[0090] By "strandedness" in the context of the monomers of the heterodimeric proteins of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities.

[0091] 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. A wide number of suitable target antigens are described below.

[0092] By "target cell" as used herein is meant a cell that expresses a target antigen.

[0093] 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.kappa., V.lamda., and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[0094] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

Heterodimeric Proteins

[0095] The present invention is directed to the generation of multispecific, particularly bispecific binding proteins, and in particular, multispecific antibodies that have one monomer comprising an scFv and the other an Fv.

Antibody

[0096] The present invention relates to the generation of multispecific antibodies, generally therapeutic antibodies. As is discussed below, the term "antibody" is used generally. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. In general, the term "antibody" includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL.

[0097] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the present invention covers pI engineering of IgG1/G2 hybrids.

[0098] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[0099] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00100] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00101] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat et al., supra (1991)).

[00102] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00103] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00104] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a

polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00105] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning."

[00106] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[0100] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[0101] It should be noted that the sequences depicted herein start at the CH1 region, position 118; the variable regions are not included except as noted. For example, the first amino acid of SEQ ID NO: 2, while designated as position"1" in the sequence listing, corresponds to position 118 of the CH1 region, according to EU numbering.

[0102] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the

numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[0103] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[0104] Another region of interest for additional substitutions, outlined below, is the Fc region. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, 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, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0105] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[0106] Alternatively, the antibodies can include a variety of structures, including, but not limited to, antibody fragments,, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[0107] In one embodiment, the antibody is an antibody fragment, as long as it contains at least one constant domain which can be engineered to produce heterodimers, such as pI engineering. Other antibody fragments that can be used include fragments that contain one or

more of the CH1, CH2, CH3, hinge and CL domains of the invention that have been pI engineered. For example, Fc fusions are fusions of the Fc region (CH2 and CH3, optionally with the hinge region) fused to another protein. A number of Fc fusions are known the art and can be improved by the addition of the heterodimerization variants of the invention. In the present case, antibody fusions can be made comprising CH1; CH1, CH2 and CH3; CH2; CH3; CH2 and CH3; CH1 and CH3, any or all of which can be made optionally with the hinge region, utilizing any combination of heterodimerization variants described herein.

[0108] In some embodiments of the present invention, one monomer comprises a heavy chain comprises a scFV linked to an Fc domain, and the other monomer comprises a heavy chain comprising a Fab linked to an Fc domain, e.g. a "typical" heavy chain, and a light chain. By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein. By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody.

Chimeric and Humanized Antibodies

[0109] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely

incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

Multispecific Antibody Constructs

[0110] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention take on a number variety of configurations, with a preferred embodiment shown in Figure 1B as a "triple F" construct.

Heterodimeric Heavy Chain Constant Regions

[0111] Accordingly, the present invention provides heterodimeric proteins based on the use of monomers containing variant heavy chain constant regions as a first domain. By "monomer" herein is meant one half of the heterodimeric protein. It should be noted that traditional antibodies are actually tetrameric (two heavy chains and two light chains). In the context of the present invention, one pair of heavy-light chains (if applicable, e.g. if the monomer comprises an Fab) is considered a "monomer". Similarly, a heavy chain region comprising the scFv is considered a monomer. In the case where an Fv region is one fusion partner (e.g. heavy and light chain) and a non-antibody protein is another fusion partner, each "half" is considered a monomer. Essentially, each monomer comprises sufficient heavy chain constant region to allow heterodimerization engineering, whether that be all the constant region, e.g. Ch1-hinge-CH2-CH3, the Fc region (CH2-CH3), or just the CH3 domain.

[0112] The variant heavy chain constant regions can comprise all or part of the heavy chain constant region, including the full length construct, CH1-hinge-CH2-CH3, or portions thereof, including for example CH2-CH3 or CH3 alone. In addition, the heavy chain region of each monomer can be the same backbone (CH1-hinge-CH2-CH3 or CH2-CH3) or different. N- and C-terminal truncations and additions are also included within the definition; for example, some pI variants include the addition of charged amino acids to the C-terminus of the heavy chain domain.

[0113] Thus, in general, one monomer of the present "triple F" construct is a scFv region-hinge-Fc domain) and the other is (VH-CH1-hinge- CH2-CH3 plus associated light chain), with heterodimerization variants, including steric and pI variants, Fc and FcRn variants, and additional antigen binding domains (with optional linkers) included in these regions.

[0114] In addition to the heterodimerization variants (e.g. steric and pI variants) outlined herein, the heavy chain regions may also contain additional amino acid substitutions, including changes for altering FcγR and FcRn binding as discussed below.

[0115] In addition, some monomers can utilize linkers between the variant heavy chain constant region and the fusion partner. For the scFv portion of the "bottle-opener", standard linkers as are known in the art can be used. In the case where additional fusion partners are made (e.g. Figures 2and 3), traditional peptide linkers can be used, including flexible linkers

of glycine and serine. In some cases, the linkers for use as components of the monomer are different from those defined below for the ADC constructs, and are in many embodiments not cleavable linkers (such as those susceptible to proteases), although cleavable linkers may find use in some embodiments.

[0116] The heterodimerization variants include a number of different types of variants, including, but not limited to, steric variants (including charge variants) and pI variants, that can be optionally and independently combined with any other variants. In these embodiments, it is important to match "monomer A" with "monomer B"; that is, if a heterodimeric protein relies on both steric variants and pI variants, these need to be correctly matched to each monomer: e.g. the set of steric variants that work (1 set on monomer A, 1 set on monomer B) is combined with pI variant sets (1 set on monomer A, 1 set on monomer B), such that the variants on each monomer are designed to achieve the desired function.

[0117] It is important to note that the heterodimerization variants outlined herein (for example, including but not limited to those variants shown in Figures 4, 5 and 6), can be optionally and independently combined with any other variants, and on any other monomer. Thus, for example, pI variants for monomer 1 from Figure 6 can be added to other heterodimerization variants for monomer 1 in Figures 5A-5B and 6 or from monomer 2. That is, what is important for the heterodimerization is that there are "sets" of variants, one set for one monomer and one set for the other. Whether these are combined from the Figures 1 to 1 (e.g. monomer 1 listings can go together) or switched (monomer 1 pI variants with monomer 2 steric variants) is irrelevant. However, as noted herein, "strandedness" should be preserved when combinations are made as outlined above. Furthermore, for the additional Fc variants (such as for FcγR binding, FcRn binding, etc.), either monomer, or both monomers, can include any of the listed variants, independently and optionally. In some cases, both monomers have the additional variants and in some only one monomer has the additional variants, or they can be combined.

Heterodimerization Variants

[0118] The present invention provides multispecific antibody formats, on a "triple F" or "bottle opener" scaffold as depicted in Figure 1[s and Figures 2 and 3]A-3E.

Steric Variants

[0119] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different

heavy chains are more likely to associate to form the heterodimeric structure than to form homodimers with the same Fc amino acid sequences. Suitable steric variants are shown in Figures 4 and 5.

[0120] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. Figures 4 and 5, further described below, identifies a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[0121] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[0122] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876, the figure and legend of which are incorporated expressly by reference herein.

[0123] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers.

pI (Isoelectric point) Variants for Heterodimers

[0124] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[0125] Preferred combinations of pI variants are shown in Figure 6, with additional pI variants disclosed in Figures 20, 21 and 22.

Heavy Chain Acidic pI Changes

[0126] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more positive (e.g. lower the pI), one or more of the following substitutions can be made: S119E, K133E, K133Q, T164E, K205E, K205Q, N208D, K210E, K210Q, K274E, K320E, K322E, K326E, K334E, R355E, K392E, a deletion of K447, adding peptide DEDE at the c-terminus, G137E, N203D, K274Q, R355Q, K392N and Q419E. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

[0127] In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

Basic pI changes

[0128] Accordingly, when one monomer comprising a variant heavy chain constant domain is to be made more negative (e.g. increase the pI), one or more of the following substitutions can be made: Q196K, P217R, P228R, N276K and H435R. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids.

Antibody Heterodimers Light chain variants

[0129] In the case of antibody based heterodimers, e.g. where at least one of the monomers comprises a light chain in addition to the heavy chain domain, pI variants can also be made in the light chain. Amino acid substitutions for lowering the pI of the light chain include, but are not limited to, K126E, K126Q, K145E, K145Q, N152D, S156E, K169E, S202E, K207E and adding peptide DEDE at the c-terminus of the light chain. Changes in this category based on the constant lambda light chain include one or more substitutions at R108Q, Q124E,