LLOREDA & CIA. S. A.
ABOGADOS

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 09-034904- -00001-0000
Fecha: 2009-05-29 17:25:59 Dep: 2020 NUECREACIONI
Tra: 11 PATENTEIYII Eve: 378 FASENACIONALI
Act: 329 CTOINFORMACION Folios: 828

Señor
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

CALLE 72 No. 5-83 5°. PISO
www.lloredacamacho.com
jllcco@lloredacamacho.com

Re: P1.-**GENENTECH, INC.**-Solicitud de Patente de Invención en Fase Nacional PCT para "**ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS**".-Clase **C07K 16/30**.-Expediente número **09-034.904.-**

1. Me refiero a mi Solicitud de Patente de Invención de 3 de Abril de 2009.

2. Atentamente me permito presentar a ese Despacho el documento que acredita el traspaso que los autores del invento de la referencia, hacen a favor de mí representado de sus derechos sobre el mismo, debidamente apostillado y junto con su Traducción Oficial No. 2009-05-11-295 debidamente legalizada hasta por el Ministerio de Relaciones Exteriores bajo el número 913742 de 12 de Mayo de 2009.

3. Así mismo, me permito presentar a usted copias debidamente certificadas de la solicitud de patente presentada para el invento arriba nombrado **respecto de la cual reclamo prioridades**, a saber, de las solicitudes estadinenses números 60/868,707, 60/863,295 y 60/921,300 de 5 de Diciembre de 2006, 27 de Octubre de 2006 y 30 de Marzo de 2007, junto con sus Traducciones Oficiales Nos. 2009-05-12-296, 2009-05-12-297 y 2009-05-12-304 correspondientes a dichos documentos, respectivamente.

4. Adicionalmente, anexo me permito presentar a usted documento completo de la Opinión Escrita de la Autoridad de Búsqueda Internacional, correspondiente a la solicitud internacional número PCT/US2007/082726, actualmente en trámite de Fase Nacional en ese despacho, bajo el número de la referencia.

5. Debidamente autorizado por mi representado, me permito manifestar a ese Despacho que es deseo del solicitante realizar modificaciones voluntarias sobre la solicitud de la referencia y, adicionalmente, es deseo del solicitante presentar en forma voluntaria un nuevo capítulo reivindicatorio que reemplaza al originalmente presentado.

6. Modificaciones al Capítulo Descriptivo

Dentro del capítulo Descriptivo se han cometido algunos errores en las referencias a las secuencias de identificación. Por lo anterior, es deseo del solicitante corregir estos errores de la siguiente manera:

a. Página 39 de la Descripción: en el parágrafo [0114] se incluyó la referencia *"(péptido revelado en SEC ID NO. 102)"* al final del mismo.
b. Página 40 de la Descripción: en el parágrafo [0119] se incluyeron las siguientes referencias: *"(SEC ID NO. 103 – 113 respectivamente en orden de aparición)"* en el aparte relativo a la Figura 17A; *"(SEC ID NO. 114 – 124 respectivamente en orden de aparición)"* en el aparte relativo a la Figura 17B; y *"(SEC ID NO. 125 – 135 respectivamente en orden de aparición)"* en el aparte relativo a la Figura 17C.
c. Página 41 de la Descripción: en el parágrafo [0124] se cambió la referencia de la secuencia de aminoácidos de SEC ID NO. 89 a SEC ID NO. 5.
d. Página 42 de la Descripción: en el parágrafo [0125] se incluyeron referencias a las secuencias de aminoácidos SEC ID NO. 136 y SEC ID NO. 137.
e. Página 182 de la Descripción: en el parágrafo [0616] se cambió la referencia de la secuencia de aminoácidos de SEC ID NO. 89 a SEC ID NO. 5.

Me permito muy atentamente señalar que dichas modificaciones no se constituyen de forma alguna en una ampliación del alcance de la invención, tal como fue originalmente presentada, toda vez que se están enmendando errores involuntarios cometidos dentro de la descripción de la invención.

7. Modificaciones al Capítulo Reivindicatorio

Me permito manifestar a ese Despacho que es deseo del solicitante presentar en forma voluntaria un nuevo capítulo reivindicatorio que reemplaza al originalmente presentado, el cual consta de 57 reivindicaciones. Este capítulo tiene como base el capítulo originalmente presentado, en el cual se han eliminado las reivindicaciones originales 7 a 24, 26, 27, 37 a 44, 50 a 55, 58, 65, 69 a 72, 74, 75, 81 a 113, 121, 122, 124, 126 a 137, 139, 143 a 151, 155 a 179, 181, 182, 184 a 203 y 205 a 224. En consecuencia, se han renumerado las reivindicaciones restantes, se han modificado las dependencias y el sustento para las nuevas reivindicaciones se encuentra en la siguiente tabla:

Nueva Reivindicación	**Sustento**
1	Reivindicación original 1 / Páginas 81 a 84 de la descripción
2	Reivindicación original 2 / Páginas 81 a 84 de la descripción
3	Reivindicación original 3 / Página 81 de la descripción
4	Reivindicación original 4

5	Reivindicación original 5
6	Reivindicación original 6
7	Reivindicación original 25
8	Reivindicación original 28
9	Reivindicación original 29
10	Reivindicación original 30
11	Reivindicación original 31
12	Reivindicación original 32
13	Reivindicación original 33
14	Reivindicación original 34
15	Reivindicación original 35
16	Reivindicación original 36
17	Reivindicación original 45
18	Reivindicación original 46
19	Reivindicación original 47
20	Reivindicación original 48
21	Reivindicación original 49
22	Reivindicación original 56
23	Reivindicación original 57
24	Reivindicación original 59
25	Reivindicación original 60
26	Reivindicación original 61 / Página 21 de la descripción
27	Reivindicaciones originales 60 y 62
28	Reivindicación original 63
29	Reivindicación original 64
30	Reivindicación original 66
31	Reivindicación original 67
32	Reivindicación original 68
33	Reivindicación original 73
34	Reivindicación original 76
35	Reivindicación original 77
36	Reivindicación original 78
37	Reivindicación original 78 y 79
38	Reivindicación original 80
39	Reivindicación original 73 y 114
40	Reivindicación original 114 y 115
41	Reivindicación original 116
42	Reivindicación original 117

43	Reivindicación original 118
44	Reivindicación original 119
45	Reivindicación original 120
46	Reivindicación original 123
47	Reivindicación original 125
48	Reivindicación original 138
49	Reivindicación original 140
50	Reivindicación original 141
51	Reivindicación original 142
52	Reivindicación original 73 y 152
53	Reivindicación original 153
54	Reivindicación original 154
55	Reivindicación original 180
56	Reivindicación original 183
57	Reivindicación original 204 / Página 81 de la descripción

En relación con la anterior modificación, atentamente me permito manifestar a ese Despacho que la misma **no amplía** de ninguna manera el alcance de la presente invención y que el nuevo capítulo modificado es el que debe ser tenido en cuenta en el trámite de Fase Nacional.

8. Por otra parte, me permito manifestar a ese Despacho que el solicitante desea modificar la Figura 12, en la cual le hace falta un residuo al péptido de la figura original.

9. Anexo me permito presentar:

a. Formulario único de modificaciones y correcciones;

b. Recibo No. 09-34,259, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de la tasa de modificación;

c. Copia de las páginas **1** a **195** que corresponden al nuevo capitulo descriptivo y al nuevo capítulo reivindicatorio que consta de **57** reivindicaciones.

d. Copia de la nueva Figura 12 en la que se ha completado el péptido con el residuo faltante, y

e. Nuevos extractos en sus dos formas, que reemplazan los originalmente presentados.

10. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,

ALICIA LLOREDA RICAURTE

T.P. 53.215

NOTARIA 41 DEL CIRCULO DE BOGOTA D.C.
DEPARTAMENTO DE CUNDINAMARCA
REPUBLICA DE COLOMBIA

El Notario 41 del circulo de Bogotá, da fe de que compareció personalmente: Alicia Lloreda Ricaurte identificada (o) con: 39690713 y tarjeta profesional de abogado No. 53215 del Consejo Superior de la Judicatura, quien autentica la firma puesta por ella (el) mismo en este escrito.
(Art. 84 C. P. C. Decreto. 2282/89 mod. 35)

Bogotá D.C. 11 MAYO 2003

EL DECLARANTE

ALICIA LLOREDA RICAURTE

C.C. No. 39.690.713 de Usaquén

NOTARIA 41 DEL CIRCULO DE BOGOTA D.C. REPUBLICA DE COLOMBIA NOTARIO 41 EN PROPIEDAD ANIBAL VILLAMIZAR CENEGO

TRADUCCION OFICIAL No. 2009-05-11-295

DE UN DOCUMENTO ESCRITO EN INGLES

APOSTILLA

(Convención de La Haya del 5 de octubre de 1961)

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por M. K. Carter
3. actuando como Funcionario que Certifica
4. lleva el sello/estampilla de la Oficina de Patentes y Marcas de los EE.UU.

Certificado

5. en Washington, D.C. 6. el 4 de mayo de 2009
7. por el Funcionario Asistente de Autenticaciones, Departamento de Estado de los Estados Unidos
8. No. 09023015-14
9. Sello/Estampilla
   Departamento de Estado
   Estados Unidos de América
10. Firma: (ilegible)
    Patrick O. Hatchett

TRADUCTORA OFICIAL
RES. 2177/89 MINJUSTICIA

ANITA ESCOBAR DE TAMAYO

LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS QUE VIEREN ESTOS PRESENTES:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

23 de abril de 2009

POR EL PRESENTE SE CERTIFICA QUE ADJUNTO AL PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS DE ESTA OFICINA DE UN DOCUMENTO REGISTRADO EL 07 de abril de 2009.

Sello del Departamento de Comercio
Oficina de Patentes y Marcas de los Estados Unidos

Por Autoridad del
Subsecretario de Comercio para Propiedad Intelectual y Director de la Oficina de Patentes y Marcas

(firma: ilegible)
M. K. CARTER
Funcionario que Certifica

TRASPASO DE PATENTE	
Versión Electrónica v1.1 04/07/2009 Versión Hoja Estilo v1.1 500829139	
TIPO DE PRESENTACION	NUEVO TRASPASO
NATURALEZA DEL TRASPASO	TRASPASO

DATOS DE LA PARTE QUE TRASPASA	
Nombre	Fecha de Ejecución
Mark S. Dennis	02/19/2009
Bonnee Rubinfeld	03/13/2009
Paul Polakis	02/22/2009
Aya Jakobovits	02/25/2009

DATOS DE LA PARTE QUE RECIBE	
Nombre:	Genentech, Inc.
Dirección:	1 DNA Way
Ciudad:	South San Francisco
Estado/País:	CALIFORNIA
Código Postal:	94080-4990

Total NUMEROS PROPIEDAD: 1	
Tipo de Propiedad	Número
Número de la Solicitud:	11978122

DATOS DE CORRESPONDENCIA

Número Fax: (650)853-10386

La correspondencia será enviada vía US Mail cuando el intento por fax no sea exitoso.

Teléfono: (650) 752-3100

Email: SKorte@goodwinprocter.com

Nombre Corresponsal: Goodwin Procter LLP

Dirección Línea 1: 135 Commonwealth Drive

Dirección Línea 4: Menlo Park, CALIFORNIA 94025

NUMERO EXPEDIENTE APODERADO	GNE-0160-R4
NOMBRE DEL PRESENTADOR:	Ginger R. Dreger
Total anexos: 8 fuente=GNE-0160-R4-Traspaso#página1.tif fuente=GNE-0160-R4-Traspaso#página2.tif fuente=GNE-0160-R4-Traspaso#página3.tif fuente=GNE-0160-R4-Traspaso#página4.tif fuente=GNE-0160-R4-Traspaso#página5.tif fuente=GNE-0160-R4-Traspaso#página6.tif fuente=GNE-0160-R4-Traspaso#página7.tif fuente=GNE-0160-R4-Traspaso#página8.tif	

TRASPASO

POR CUANTO, AMRK S. DENNIS, un ciudadano de los ESTADOS UNIDOS DE AMERICA, residente en 120 PLYMOUTH, SAN CARLOS, CA 94070; BONNEE RUBINFELD, un ciudadano de los ESTADOS UNIDOS DE AMERICA, residente en 4186 QUAIL RUN DRIVE, DANVILLE, CALIFORNIA 94506; PAUL POLAKIS, un ciudadano de los ESTADOS UNIDOS DE AMERICA, residente en 265 WEST BLITHEDALE AVENUE, MILL VALLEY CALIFORNIA 94941; Y AYA JAKOBOVITS, un ciudadano de los ESTADOS UNIDOS DE AMERICA, residente en 3135 HUTTON DRIVE, BEVERLY HILLS, CALIFORNIA 90210 (en adelante "CEDENTES"), hemos inventado una invención nueva y útil en

ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

para la cual una solicitud Serie No. 11/978.122, ha sido radicada por ellos el 26 de octubre de 2007; y

POR CUANTO, GENENTECH, INC. una sociedad constituida y existente bajo y en virtud de las leyes del Estado de Delaware, que tiene un lugar de negocios en 1 DNA Way, South San Francisco, California 94080-4990, desea adquirir un interés en y de dicha invención, y en y de Patente de Invención que se obtendrá por lo tanto;

AHORA, POR LO TANTO, sépase por todos a quienes corresponda;

Que por una contraprestación buena y valiosa el recibo de lo cual se reconoce por el presente, dichos CEDENTES han y por el presente venden, ceden, transfieren y traspasas a dicho GENENTECH, INC. sus sucesores y cesionarios, todo el derecho, título e interés incluyendo todos los derechos bajo la Convención de París para la Protección de Propiedad

Industrial, en y de dicha invención, y en y de cualquiera y todas las Patentes de Invención que se otorguen y expidan por lo tanto o cualquier continuación, división, renovación o sustituto de ello, y en cuanto a Patente de Invención cualquier reexpedición o reexamen de ello, no únicamente para, de y en los Estados Unidos de América, sus territorios y posesiones, pero para, de y en todos los otros países; y ha sido y por el presente se autoriza y solicita que las agencias gubernamentales apropiadas expidan dicha Patente de Invención a GENENTECH, INC., de conformidad con este Traspaso.

Dichos CEDENTES acuerdan y consienten cooperar con GENENTECH, INC., para permitirle a dicho GENENTECH, INC. disfrutar todo el alcance el derecho, título e interés por el presente traspasado en los Estados Unidos y países extranjeros. Dicha cooperación por dichos CEDENTES incluye la producción oportuna de hechos y documentos pertinentes, dar testimonio, ejecución de memoriales, juramentos, especificaciones, declaraciones u otros documentos, y otra asistencia todo del alcance considerado o deseable por dicho GENENTECH, INC., (a) para perfeccionar el derecho, título e interés por el presente traspasado; (b) para procesar cualquiera de dichas solicitudes; (c) para radicar y procesar solicitudes para reexpedición de cualquiera de dichas patentes; (d) para interferencia o otros procedimientos de prioridad que comprendan a dicha invención; y (e) para procedimientos legales que comprendan a dicha invención y cualquier solicitud por lo tanto y cualquier patente otorgada sobre ello, incluyendo sin limitación procedimientos de oposición, procedimientos de cancelación, impugnaciones de prioridad, procedimientos de uso público, acciones de infracción y acciones de tribunal; siempre y cuando, sin embargo, que el gasto incurrido por dicho CEDENTE para proporcionar dicha cooperación sea pagado por dicho GENETECH, INC.

Los términos y acuerdos de este traspaso entrarán en vigencia para el beneficio de dicho GENENTECH, INC., sus sucesores, cesionarios y otros representantes legales, y será obligatorio para dicho CEDENTE, sus respectivos herederos, representantes legales y cesionarios.

Dichos CEDENTES por el presente garantizan y afirman que no han celebrado y no celebrarán ningún traspaso, contrato o entendimiento en conflicto con el presente.

EN TESTIMONIO DE LO CUAL, firmamos como sigue:

SAN CARLOS	(firma: ilegible)
Fechado: 2/19/09	MARK S. DENNIS
DANVILLE	(firma: ilegible)
Fechado: 3/13/09	BONNEE RUVINGELD
MILL VALLEY	(firma: ilegible)
Fechado: 2/22/09	PAUL POLAKIS
BEVERLY HILLS	(firma: ilegible)
Fechado: 2/25/09	AYA JAKOBOVITS[1]

Traducido según mi mejor saber y entender, este día 11 de mayo de 2009, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTORA OFICIAL
RES. 2177/89 MINJUSTICIA
ANITA ESCOBAR DE TAMAYO

[1] Nota del Traductor: Se repite el documento de traspaso con las firmas de cada traductor en notas aparte.

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country: *United States of America*

This public document

2. has been signed by M.K. Carter

3. acting in the capacity of Certifying Officer

4. bears the seal/stamp of U.S. Patent and Trademark Office

Certified

5. at Washington, D.C.

6. the fourth of May, 2009

7. by *Assistant Authentication Officer, United States Department of State*

8. No. 09023015-14

9. Seal/Stamp:

10. Signature:

Patrick O. Hatchett

A 1750565

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

April 23, 2009

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF A DOCUMENT RECORDED ON APRIL 07, 2009.

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

M. K. CARTER
Certifying Officer

PATENT ASSIGNMENT

Electronic Version v1.1 04/07/2009
Stylesheet Version v1.1 500829139

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Mark S. Dennis	02/19/2009
Bonnee Rubinfeld	03/13/2009
Paul Polakis	02/22/2009
Aya Jakobovits	02/25/2009

RECEIVING PARTY DATA

Name:	Genentech, Inc.
Street Address:	1 DNA Way
City:	South San Francisco
State/Country:	CALIFORNIA
Postal Code:	94080-4990

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	11978122

CH $40.00 11978122

CORRESPONDENCE DATA

Fax Number: (650)853-1038
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: (650) 752-3100
Email: SKorte@goodwinprocter.com
Correspondent Name: Goodwin Procter LLP
Address Line 1: 135 Commonwealth Drive
Address Line 4: Menlo Park, CALIFORNIA 94025

ATTORNEY DOCKET NUMBER:	GNE-0160-R4
NAME OF SUBMITTER:	Ginger R. Dreger

Total Attachments: 8
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source=GNE-0160-R4-Assignment#page8.tif

ASSIGNMENT

WHEREAS, **MARK S. DENNIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **120 PLYMOUTH, SAN CARLOS, CA 94070 ; BONNEE RUBINFELD**, a citizen of **UNITED STATES OF AMERICA**, residing at **4186 QUAIL RUN DRIVE, DANVILLE, CALIFORNIA 94506; PAUL POLAKIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **265 WEST BLITHEDALE AVENUE, MILL VALLEY, CALIFORNIA 94941**; and **AYA JAKOBOVITS**, a citizen of **UNITED STATES OF AMERICA**, residing at **3135 HUTTON DRIVE, BEVERLY HILLS, CALIFORNIA 90210** (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THERFOR

for which an application Serial No. **11/978,122**, filed on **October 26, 2007**, for Letters Patent has been by them on ; and

WHEREAS, GENENTECH, INC., a corporation organized and existing under and by virtue of the laws of the State of Delaware, having a place of business at 1 DNA Way, South San Francisco, California 94080-4990, is desirous of acquiring an interest in and to said invention, and in and to the Letters Patents to be obtained therefor;

NOW, THEREFORE, be it known by all whom it may concern;

That for good and valuable consideration the receipt of which is hereby acknowledged, the said ASSIGNORS have and do hereby sell, assign, transfer and set over unto the said GENENTECH, INC., its successors and assigns, the full and exclusive right, title and interest including all rights under the Paris Convention for the Protection of Industrial Property, in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor or any continuation, division, renewal, or substitute thereof, and as to Letters Patents any reissue or re-examination thereof, not only for, to, and in the United States of America, its territories and possessions, but for, to and in all other countries; and it has been and is hereby authorized and requested that the appropriate government agencies issue said Letters Patents to said GENENTECH, INC., in accordance with this Assignment.

Said ASSIGNORS covenant and agree to cooperate with GENENTECH, INC., to enable said GENENTECH, INC. to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said ASSIGNORS includes prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said GENENTECH, INC., (a) for perfecting the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting applications for reissuance of any of said patents; (d) for interference or other priority proceedings involving said invention; and (e) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said ASSIGNORS in providing such cooperation shall be paid for by said GENENTECH, INC.

REEL 022515 FRAME 0860

The terms and covenants of this assignment shall inure to the benefit of said GENENTECH, INC., its successors, assigns and other legal representatives, and shall be binding upon said ASSIGNORS, their respective heirs, legal representatives and assigns.

Said ASSIGNORS hereby warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF we undersign as follows;

SAN CARLOS
Dated: 2/19/09

MARK S. DENNIS

DANVILLE
Dated: _____

BONNEE RUBINFELD

MILL VALLEY
Dated: _____

PAUL POLAKIS

BEVERLY HILLS
Dated: _____

AYA JAKOBOVITS

ASSIGNMENT

WHEREAS, **MARK S. DENNIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **120 PLYMOUTH, SAN CARLOS, CA 94070 ; BONNEE RUBINFELD**, a citizen of **UNITED STATES OF AMERICA**, residing at **4186 QUAIL RUN DRIVE, DANVILLE, CALIFORNIA 94506; PAUL POLAKIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **265 WEST BLITHEDALE AVENUE, MILL VALLEY, CALIFORNIA 94941**; and **AYA JAKOBOVITS**, a citizen of **UNITED STATES OF AMERICA**, residing at **3135 HUTTON DRIVE, BEVERLY HILLS, CALIFORNIA 90210** (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THERFOR

for which an application Serial No. **11/978,122**, filed on **October 26, 2007**, for Letters Patent has been by them on ; and

WHEREAS, GENENTECH, INC., a corporation organized and existing under and by virtue of the laws of the State of Delaware, having a place of business at 1 DNA Way, South San Francisco, California 94080-4990, is desirous of acquiring an interest in and to said invention, and in and to the Letters Patents to be obtained therefor;

NOW, THEREFORE, be it known by all whom it may concern;

That for good and valuable consideration the receipt of which is hereby acknowledged, the said ASSIGNORS have and do hereby sell, assign, transfer and set over unto the said GENENTECH, INC., its successors and assigns, the full and exclusive right, title and interest including all rights under the Paris Convention for the Protection of Industrial Property, in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor or any continuation, division, renewal, or substitute thereof, and as to Letters Patents any reissue or re-examination thereof, not only for, to, and in the United States of America, its territories and possessions, but for, to and in all other countries; and it has been and is hereby authorized and requested that the appropriate government agencies issue said Letters Patents to said GENENTECH, INC., in accordance with this Assignment.

Said ASSIGNORS covenant and agree to cooperate with GENENTECH, INC., to enable said GENENTECH, INC. to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said ASSIGNORS includes prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said GENENTECH, INC., (a) for perfecting the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting applications for reissuance of any of said patents; (d) for interference or other priority proceedings involving said invention; and (e) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said ASSIGNORS in providing such cooperation shall be paid for by said GENENTECH, INC.

The terms and covenants of this assignment shall inure to the benefit of said GENENTECH, INC., its successors, assigns and other legal representatives, and shall be binding upon said ASSIGNORS, their respective heirs, legal representatives and assigns.

Said ASSIGNORS hereby warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF we undersign as follows;

SAN CARLOS
Dated: _____

_____
MARK S. DENNIS

DANVILLE
Dated: 3/13/09

Bonnee Rubinfeld
BONNEE RUBINFELD

MILL VALLEY
Dated: _____

_____
PAUL POLAKIS

BEVERLY HILLS
Dated: _____

_____
AYA JAKOBOVITS

ASSIGNMENT

WHEREAS, **MARK S. DENNIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **120 PLYMOUTH, SAN CARLOS, CA 94070** ; **BONNEE RUBINFELD**, a citizen of **UNITED STATES OF AMERICA**, residing at **4186 QUAIL RUN DRIVE, DANVILLE, CALIFORNIA 94506; PAUL POLAKIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **265 WEST BLITHEDALE AVENUE, MILL VALLEY, CALIFORNIA 94941**; and **AYA JAKOBOVITS**, a citizen of **UNITED STATES OF AMERICA**, residing at **3135 HUTTON DRIVE, BEVERLY HILLS, CALIFORNIA 90210** (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THERFOR

for which an application Serial No. **11/978,122**, filed on **October 26, 2007**, for Letters Patent has been by them on ; and

WHEREAS, GENENTECH, INC., a corporation organized and existing under and by virtue of the laws of the State of Delaware, having a place of business at 1 DNA Way, South San Francisco, California 94080-4990, is desirous of acquiring an interest in and to said invention, and in and to the Letters Patents to be obtained therefor;

NOW, THEREFORE, be it known by all whom it may concern;

That for good and valuable consideration the receipt of which is hereby acknowledged, the said ASSIGNORS have and do hereby sell, assign, transfer and set over unto the said GENENTECH, INC., its successors and assigns, the full and exclusive right, title and interest including all rights under the Paris Convention for the Protection of Industrial Property, in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor or any continuation, division, renewal, or substitute thereof, and as to Letters Patents any reissue or re-examination thereof, not only for, to, and in the United States of America, its territories and possessions, but for, to and in all other countries; and it has been and is hereby authorized and requested that the appropriate government agencies issue said Letters Patents to said GENENTECH, INC., in accordance with this Assignment.

Said ASSIGNORS covenant and agree to cooperate with GENENTECH, INC., to enable said GENENTECH, INC. to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said ASSIGNORS includes prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said GENENTECH, INC., (a) for perfecting the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting applications for reissuance of any of said patents; (d) for interference or other priority proceedings involving said invention; and (e) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said ASSIGNORS in providing such cooperation shall be paid for by said GENENTECH, INC.

The terms and covenants of this assignment shall inure to the benefit of said GENENTECH, INC., its successors, assigns and other legal representatives, and shall be binding upon said ASSIGNORS, their respective heirs, legal representatives and assigns.

Said ASSIGNORS hereby warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF we undersign as follows;

SAN CARLOS
Dated: _____

_____
MARK S. DENNIS

DANVILLE
Dated: _____

_____
BONNEE RUBINFELD

MILL VALLEY
Dated: 2-22-09

_____
PAUL POLAKIS

BEVERLY HILLS
Dated: _____

_____
AYA JAKOBOVITS

ASSIGNMENT

WHEREAS, **MARK S. DENNIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **120 PLYMOUTH, SAN CARLOS, CA 94070 ; BONNEE RUBINFELD**, a citizen of **UNITED STATES OF AMERICA**, residing at **4186 QUAIL RUN DRIVE, DANVILLE, CALIFORNIA 94506; PAUL POLAKIS**, a citizen of **UNITED STATES OF AMERICA**, residing at **265 WEST BLITHEDALE AVENUE, MILL VALLEY, CALIFORNIA 94941**; and **AYA JAKOBOVITS**, a citizen of **UNITED STATES OF AMERICA**, residing at **3135 HUTTON DRIVE, BEVERLY HILLS, CALIFORNIA 90210** (hereinafter "ASSIGNORS"), have invented a new and useful invention in

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NOW, THEREFORE, be it known by all whom it may concern;

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SAN CARLOS
Dated: _____

_____
MARK S. DENNIS

DANVILLE
Dated: _____

_____
BONNEE RUBINFELD

MILL VALLEY
Dated: _____

_____
PAUL POLAKIS

BEVERLY HILLS
Dated: 2/25/09

Aya Jakobovits
AYA JAKOBOVITS

Application Serial No.: 11/978,122
Attorney Docket No.: GNE-0160-R4

LIBC/3499983.1

RECORDED 04/07/2009

REEL 022515 FRAME 0867

TRADUCCION OFICIAL No. 2009-05-12-296
DE UN DOCUMENTO ESCRITO EN INGLES

LOS ESTADOS UNIDOS DE AMERICA
A TODOS AQUELLOS QUE VIEREN ESTOS PRESENTES:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas de los Estados Unidos

09 de abril de 2009

POR EL PRESENTE SE CERTIFICA QUE ADJUNTO AL PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS DE AQUELLOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE CUMPLIO CON LOS REQUISITOS PARA QUE SE LE OTORGUE UNA FECHA DE PRESENTACION BAJO 35 USC 111.

NUMERO DE SOLICITUD: *60/868.707*
FECHA DE PRESENTACION: *05 de diciembre de 2006*

EL CODIGO DEL PAIS Y EL NUMERO DE SU SOLICITUD DE PRIORIDAD, PARA SER USADOS PARA PRESENTACION EN EL EXTERIOR BAJO LA CONVENCION DE PARIS, *ES US60/868.707*

Sello del Departamento de Comercio
Oficina de Patentes y Marcas de los Estados Unidos

Por Autoridad del
Subsecretario de Comercio para Propiedad Intelectual y Director de la Oficina de Patentes y Marcas de los Estados Unidos

(firma: ilegible)

E. BORNETT

Funcionario que Certifica

PRESENTADA ELECTRONICAMENTE EL: 5 DE DICIEMBRE DE 2006

CARATULA DE SOLICITUD PROVISIONAL

Esta es una solicitud para presentar una SOLICITUD PROVISONAL bajo 37 C.F.R. 1.53(c).

EXPEDIENTE NUMERO	PR-2412-1	Marque un signo más (+) dentro de esta casilla →	+

INVENTOR(es)/SOLICITANTE(s)			
APELLIDO	NOMBRE	SEGUNDO NOMBRE	RESIDENCIA (CIUDAD Y ESTADO O PAIS EXTRANJERO)
1) Dennis	Mark		South San Francisco, CA
2) Rubinfeld	Bonee		South San Francisco, CA
3) Polakis	Paul		South San Francisco, CA
4) Jakobovits	Aya		Santa Monica, CA

IDENTIFICADOR DE LA SOLICITUD (si no se nombra ningún INVENTOR(s)/SOLICITANTE(s)					
TITULO DE LA INVENCION *(280 caracteres max)*					
ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS					
DIRECCION DE CORRESPONDENCIA					
CLIENTE NO. 09157			Número de Teléfono (650) 225-2066		
			Número de Fax (650) 952-9881		
ESTADO	California	CODIGO POSTAL	94080-4990	PAIS	EU
PARTES DE LA SOLICITUD ADJUNTAS Y CALCULO DE DERECHOS					

Número Presentado		Número Extra		Tasa	Derechos Básicos 37 CFR 1.16(a)
					$200
Total Páginas (Incluyendo Figuras y Listado de Secuencia impreso, ya sea registrado con o sin un diskette/CD)	178	- 100 =	78	$250	$500.00
DERECHO DE PRESENTACION PROVISIONAL TOTAL					$700.00

METODO DE PAGO (*marque uno)*

☒ Se adjunta un cheque o giro postal para cubrir los derechos de presentación Provisional.

☐ Por el presente el Comisionado está autorizado para cargar derechos de presentación y acreditar Cuenta de Depósito No. 07-0630. Por el presente el Comisionado está autorizado para cargar cualquier derecho adicional requerido bajo 37 CFR §1.16 y 1.17, o acreditar sobrepago a la Cuenta de Depósito No. 07-0630. Se adjunta un duplicado de esta hoja.

La invención fue hecha por una agencia del gobierno de los Estados Unidos o bajo un contrato con una agencia del gobierno de los Estados Unidos.

☒ No.

☐ Sí, el nombre de la agencia del gobierno de los EU y el número(s) del contrato del gobierno es(son):

Presentado respetuosamente,

GENENTECH, INC.

Fecha: 5 de diciembre de 2006 Por: (firma: ilegible)

Deirdre L. Conley, Ph.D.

Reg. No. 36.487

Teléfono No. (650) 225-2066

Recibo de Reconocimiento Electrónico	
ID EFS:	1354268
Número de Solicitud:	60868707
Número de Solicitud Internacional:	
Número de Confirmación:	7139
Título de la Invención:	ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS
Primer Inventor Nombrado/Nombre del Solicitante:	Mark Dennis
Número del Cliente:	09157
Registrador:	Deirdre L. Conley
Registrador Autorizado por:	
Número de Expediente del Apoderado:	PR2412-1
Fecha de Recepción:	05-DEC-2006
Fecha de Presentación:	
Estampilla de Tiempo:	19:41:34
Tipo de Solicitud:	Provisional
Información de Pago:	
Presentada con Pago	sí
El pago fue exitosamente recibido en RAM	$450
Número confirmación RAM	850
Cuenta de Depósito	070630
El Director de la USPTO por el presente está autorizado para cobrar los derechos indicados y cualquier sobre pago así: Cobrar cualquier Derecho Adicional requerido bajo 37 C.F.R. Sección 1.16 y 1.17	

Listas de Archivos:

Número del Documento	Descripción del Documento	Nombre del Archivo	Tamaño del Archivo (Bytes)	Multi Partes/.zip	Páginas (si aplica)
1		PR2412-1_SOLICITUD_PROVISIONAL.pdf	8998616	sí	159

Descripción Multipartes/archivo PDF en descripción .zip

Descripción del Documento	Inicio	Fin
Especificación	1	151
Reivindicaciones	152	158
Resumen	159	159

Advertencias:

Información:

2	Dibujos	PR2412-1_DIBUJOS.pdf	761893	no	19

Advertencias:

Información:

3	Transmisión de Nueva Solicitud	PR2412-1_SOLICITUD_PROVISIONAL_CARATULA.pdf	47686	no	1

Advertencias:

Información:

4	Hoja de Trabajo de Derechos (PTO-875)	Fee-info.pdf	8261	no	2

Advertencias:

Información:	
Tamaño Total Archivos (en bytes):	9816456

Este Recibo de Reconocimiento evidencia recibo en la fecha anotada por la USPTO de los documentos indicados, caracterizados por el solicitante, e incluyendo conteo de páginas, donde aplique. Sirve como evidencia del recibo similar a una Tarjeta Postal, según se describe en MPEP 503.

Nuevas Solicitudes Bajo 35 U.S.C. 111

Si se presenta una nueva solicitud y la solicitud incluye los componentes necesarios para una fecha de presentación (ver 37 CFR 1.53(b)-(d) y MPEP 506), un Recibo de Presentación (37 CFR 1.54) será expedido en el curso debido y la fecha mostrada en este Recibo de Reconocimiento establecerá la fecha de presentación de la solicitud.

Fase Nacional de una Solicitud Internacional bajo 35 U.S.C. 371

Si una presentación a tiempo para registrar una solicitud internacional en la fase nacional cumple con las condiciones de 35 U.S.C. 371 y otros requerimientos que aplican se expedirá un Formato PCT/DO/EO/903 que indica aceptación de la solicitud como una presentación de la fase nacional bajo 35 U.S.C. 371 además del Recibo de Presentación, en su curso debido.

Traducido según mi mejor saber y entender, este día 12 de mayo de 2009, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

PA 7178470

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 09, 2009

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/868,707***
FILING DATE: ***December 05, 2006***

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS ***US60/868,707***

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

E. BORNETT
Certifying Officer

FILED ELECTRONICALLY ON: 5 DECEMBER 2006

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

DOCKET NUMBER	PR2412-1	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
1) Dennis	Mark		South San Francisco, CA
2) Rubinfeld	Bonee		South San Francisco, CA
3) Polakis	Paul		South San Francisco, CA
4) Jakobovits	Aya		Santa Monica, CA

APPLICATION IDENTIFIER (if no INVENTOR(s)/APPLICANT(s) are named)

TITLE OF THE INVENTION *(280 characters max)*

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

CORRESPONDENCE ADDRESS

CUSTOMER NO: 09157			Telephone Number (650) 225-2066		
			Facsimile Number (650) 952-9881		
STATE	California	ZIP CODE	94080-4990	COUNTRY	USA

ENCLOSED APPLICATION PARTS AND FEE CALCULATION

	Number Filed		Number Extra		Rate	Basic Fee 37 CFR 1.16(a)
						$200
Total Pages (Including Figures and printed Sequence Listing, whether filed with or without a diskette/CD)	178	- 100 =	78		$250	$500.00
				TOTAL PROVISIONAL FILING FEE		$700.00

METHOD OF PAYMENT *(check one)*

☐ A check or money order is enclosed to cover the Provisional filing fees.

☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 07-0630. The Commissioner is hereby authorized to charge any additional fees required under 37 CFR §1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. A duplicate of this sheet is enclosed.

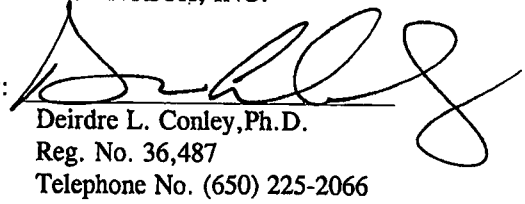
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number(s) is(are):

Respectfully submitted,
GENENTECH, INC.

Date: December 5, 2006

By: 

Deirdre L. Conley,Ph.D.
Reg. No. 36,487
Telephone No. (650) 225-2066

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Electronic Acknowledgement Receipt

EFS ID:	1354268
Application Number:	60868707
International Application Number:	
Confirmation Number:	7139
Title of Invention:	ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR
First Named Inventor/Applicant Name:	Mark Dennis
Customer Number:	09157
Filer:	Deirdre L. Conley
Filer Authorized By:	
Attorney Docket Number:	PR2412-1
Receipt Date:	05-DEC-2006
Filing Date:	
Time Stamp:	19:41:34
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$450
RAM confirmation Number	850
Deposit Account	070630
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 and 1.17	

File Listing:

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Document Number	Document Description	File Name	File Size(Bytes)	Multi Part /.zip	Pages (if appl.)
1		PR2412-1_PROVISIONAL_APPLICATION.pdf	8998616	yes	159

Multipart Description/PDF files in .zip description		
Document Description	**Start**	**End**
Specification	1	151
Claims	152	158
Abstract	159	159

Warnings:

Information:

2	Drawings	PR2412-1_DRAWINGS.pdf	761893	no	19

Warnings:

Information:

3	Transmittal of New Application	PR2412-1_PROVISIONAL_APPLICATION_COVERSHEET.pdf	47686	no	1

Warnings:

Information:

4	Fee Worksheet (PTO-875)	fee-info.pdf	8261	no	2

Warnings:

Information:

Total Files Size (in bytes):	9816456

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

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

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

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ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates to anti-STEAP-1 antibodies and immunconjugates thereof. The invention further relates to methods of using anti-STEAP-1 antibodies and immunconjugates thereof.

BACKGROUND

FIELD OF THE INVENTION

The present invention relates to anti-STEAP-1 antibody compositions and methods of killing STEAP-1 expressing cancers cells.

BACKGROUND OF THE INVENTION

In humans, prostate cancer is one of the most commonly diagnosed malignancies in males and is the second leading cause of cancer related death in men. The American Cancer Society estimates that for the year 2000, 180,400 new cases of prostate cancer will be diagnosed with 31,900 deaths from the disease. In advanced stages, prostate cancer metastasizes to the bone. While advances in early diagnosis and treatment of locally confined tumors have been achieved, prostate cancer is incurable once it has metastasized. Patients with metastatic prostate cancer on hormonal therapy will eventually develop an androgen-refractory (androgen independent) state that will lead to disease progression and death. Currently, prostate-specific antigen (PSA) is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer. However, widespread use of PSA as a tool for screening is controversial since PSA fails to discriminate accurately between benign and malignant prostate disease.

Depending on the stage of the cancer, prostate and bladder cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, chemotherapy, androgen deprivation (e.g., hormonal therapy) in the case of prostate cancer. While surgical or radiation therapy significantly improves survival in patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences following hormone ablation. The majority of patients who undergo hormone therapy progress to develop androgen-independent disease. Currently, there is no effective treatment for the 20-40% of prostate cancer patients who develop recurrent disease after surgery or radiation therapy, or for those in whom the cancer has

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metastasized at the time of diagnosis. Chemotherapy has its toxic side effects, especially in elderly patients. Development of new forms of therapy especially for disease refractory to androgen deprivation is an urgent need in the management of prostatic carcinoma.

The identification of a novel cell surface antigen, STEAP-1 has been described (see US6,329,503). STEAP-1 is member of cell surface serpentine transmembrane antigens. It is expressed predominantly in the prostate cancer, and thus members of this family have been tered "STEAP" (Six Transmembrane Epithelial Antigens of the Prostate). Human STEAP proteins exhibit a high degree of structural conservation within the family by show no significant structural homology to any known human proteins. STEAP-1 appears to be a type IIIa membrane protein expressed predominantly in prostate cells in normal human tissues. Structurally, STEAP-1 is a 339 amino acid protein characterized by a molecular topology of six transmembrane domains and intracellular N- and C-termini, suggesting that it folds in a "serpentine" manner into three extracellular and two intracellular loops. STEAP-1 protein expression is maintained at high levels across various states of prostate cancer. STEAP-1 is highly over-expressed in other human cancers such as lung and colon. Murine antibodies have been raised to human STEAP-1 fragments and the antibodies were shown to bind STEAP-1 on the cell surface (see US20040253232A1).

Antibody-based therapy has proved very effective in the treatment of various cancers. For example, HERCEPTIN® and RITUXAN® (both from Genentech, S. San Francisco), have been used successfully to treat breast cancer and non-Hodgkin's lymphoma, respectively. HERCEPTIN® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) proto-oncogene. HER2 protein overexpression is observed in 25-30% of primary breast cancers. RITUXAN® is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. Both these antibodies are produced in CHO cells.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4975278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin,

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methotrexate, and vindesine (Rowland et al., Cancer Immunol. Immunother. 21:183-87 (1986)). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Kerr et al (1997) Bioconjugate Chem. 8(6):781-784; Mandler et al (2000) Journal of the 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). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition (Meyer, D.L. and Senter, P.D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" in Annual Reports in Medicinal Chemistry, Vol 38 (2003) Chapter 23, 229-237). Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is being developed for the treatment of cancers that express CanAg antigen, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The same maytansinoid drug moiety, DM1, was linked through a non-disulfide linker, SMCC, to a mouse murine monoclonal antibody, TA.1 (Chari et al. (1992) Cancer Research 52:127-131). This conjugate was reported to be 200-fold less potent than the corresponding disulfide linker conjugate. The SMCC linker was considered therein to be "noncleavable."

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Several short peptidic compounds have been isolated from the marine mollusk, Dolabella auricularia, and found to have biological activity (Pettit et al (1993) Tetrahedron 49:9151; Nakamura et al (1995) Tetrahedron Letters 36:5059-5062; Sone et al (1995) Journal Org Chem. 60:4474). Analogs of these compounds have also been prepared, and some were found to have biological activity (for a review, see Pettit et al (1998) Anti-Cancer Drug Design 13:243-277). For example, auristatin E (US 5635483) is a synthetic analogue of the marine natural product Dolastatin 10, an agent that inhibits tubulin polymerization by binding to the same site on tubulin as the anticancer drug vincristine (G. R. Pettit, (1997) Prog. Chem. Org. Nat. Prod. 70:1-79). Dolastatin 10, auristatin PE, and auristatin E are linear peptides having four amino acids, three of which are unique to the dolastatin class of compounds, and a C-terminal amide.

The auristatin peptides, auristain E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to: (i) chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas); (ii) cAC10 which is specific to CD30 on hematological malignancies (Klussman, et al (2004), Bioconjugate Chemistry 15(4):765-773; Doronina et al (2003) Nature Biotechnology 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco et al (2003) Blood 102(4):1458-1465; US 2004/0018194; (iii) anti-CD20 antibodies such as Rituxan® (rituximab) (WO 04/032828) for the treatment of CD20-expressing cancers and immune disorders; (iv) anti-EphB2 antibodies 2H9 and anti-IL-8 for treatment of colorectal cancer (Mao, et al (2004) Cancer Research 64(3):781-788); (v) E-selectin antibody (Bhaskar et al (2003) Cancer Res. 63:6387-6394); and (vi) other anti-CD30 antibodies (WO 03/043583). Monomethylauristatin (MMAE) has also been conjugated to 2H9, an antibody against EphB2R which is a type 1 TM tyrosine kinase receptor with close homology between mouse and human, and is over-expressed in colorectal cancer cells (Mao et al (2004) Cancer Res. 64:781-788).

Monomethylauristatin MMAF, a variant of auristatin E (MMAE) with a phenylalanine at the C-terminus (US 5767237; US 6124431), has been reported to be less potent than MMAE, but more potent when conjugated to monoclonal antibodies (Senter et al, Proceedings fo the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004). Auristatin F phenylene diamine (AFP); a phenylalanine variant of MMAE was linked to an anti-CD70 mAb, 1F6, through the C-terminus of 1F6 via a phenylene diamine spacer (Law et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 625, presented March 28, 2004).

There exists a need in the art for additional drugs to treat various cancers such as cancers and metatases of cancers in the prostate, lung and colon. Particularly useful drugs for this purpose include

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prostate, lung or colon cell targeted anti-STEAP-1 antibody-drug conjugates having a significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

The recitation of any reference in this application is not an admission that the reference is prior art to this application. All references cited herein, including patents, patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

The invention provides anti-STEAP-1 antibodies and methods of using the same.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) or a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9). In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) and a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9).

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79.

In one aspect, the antibody comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of

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SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79. In an embodiment, the antibody further comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In certain embodiments, a polynucleotide encoding any of the above antibodies is provided. In one embodiment, a vector comprising the polynucleotide is provided. In one embodiment, a host cell comprising the vector is provided. In one embodiment, the host cell is eukaryotic. In one embodiment, the host cell is a Chinese hamster ovary (CHO) cell. In one embodiment, a method of making an anti-STEAP-1 antibody is provided, wherein the method comprises culturing the host cell under conditions suitable for expression of the polynucleotide encoding the antibody, and isolating the antibody.

In one aspect, an antibody that binds to STEAP-1 expressed on the surface of a cell is provided. In one embodiment, the antibody binds to an epitope within a region of human or mouse STEAP-1. In one embodiment, the cell is mammalian cell. In one embodiment, the cell is a human cell. In one embodiment, the cell is a cancer cell. In one embodiment the cell is a prostate, lung or colon cell. In one embodiment the cancer cell is a prostate cancer cell. In another embodiment, the cell cell from a metastisis of a primary prostate, lung or colon cancer.

In certain embodiments, any of the above antibodies is a monoclonal antibody. In one embodiment, the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one embodiment, the antibody is humanized. In one embodiment, the antibody is human.

In one aspect, a method of detecting the presence of STEAP-1 in a biological sample is provided, the method comprising contacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1. In one embodiment, the biological sample comprises prostate cells. In one embodiment, the biological sample is from a mammal experiencing or suspected of experiencing a prostate cell disorder and/or a cell proliferative disorder of cells or tissues including, but not limited to, prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma as well as metastases of primary prostate, lung, colon, bladder, and ovarian cancers and Ewing's sarcoma. See, for example, (see US6,329,503; and Rodeberg, D.A. et al., Clin. Cancer Res. 11(12):4545-4552 (2005)).

In one aspect, a method of diagnosing a cell proliferative disorder associated with increased expression of STEAP-1 is provided, the method comprising contacting a test cell with any of the above

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antibodies; determining the level of expression of STEAP-1 by detecting binding of the antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell, wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a cell proliferative disorder associated with increased expression of STEAP-1. In one embodiment, the test cell is a cell from a patient suspected of having a cell proliferative disorder, such as a prostate proliferative disorder. In one embodiment, the cell proliferative disorder is selected from prostate cell disorders including but not limited to prostate cancer. In one embodiment, the method comprises determining the level of expression of STEAP-1 on the surface of the test cell (such as, for example, a prostate cancer cell) and comparing the level of expression of STEAP-1 on the surface of the test cell with the level of expression of STEAP-1 on the surface of the control cell (such, for example, as a normal prostate cell other than an abnormally proliferating prostate cell).

In one aspect, a method of diagnosing a cell proliferative disorder associated with an increase in cells, such as prostate cells, expressing STEAP-1 is provided, the method comprising contacting a test cells in a biological sample with any of the above antibodies; determining the level of antibody bound to test cells in the sample by detecting binding of the antibody to STEAP-1; and comparing the level of antibody bound to cells in a control sample, wherein the level of antibody bound is normalized to the number of STEAP-1-expressing cells in the test and control samples, and wherein a higher level of antibody bound in the test sample as compared to the control sample indicates the presence of a cell proliferative disorder associated with cells expressing STEAP-1.

In one aspect, a method of detecting soluble STEAP-1 in blood or serum, the method comprising contacting a test sample of blood or serum from a mammal suspected of experiencing a prostate cell proliferative disorder with an anti-STEAP-1 antibody of the invention and detecting a increase in soluble STEAP-1 in the test sample relative to a control sample of blood or serum from a normal mammal. In an embodiment, the method of detecting is useful as a method of diagnosing a prostate cell proliferative disorder associated with an increase in soluble STEAP-1 in blood or serum of a mammal.

In one aspect, the antibodies of the invention include cysteine engineered antibodies where one or more amino acids of a parent antibody are replaced with a free cysteine amino acid as disclosed in WO2006/034488 (herein incorporated by reference in its entirety). A cysteine engineered antibody comprises one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0. A free cysteine amino acid is a cysteine residue which has been engineered into the parent antibody and is not part of a disulfide bridge. Cysteine engineered antibodies are useful for attachment of cytotoxic and/or imaging compounds at the site of the engineered cysteine through, for example, a maleimide or haloacetyl.

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The nucleophilic reactivity of the thiol functionality of a Cys residue to a maleimide group is about 1000 times higher compared to any other amino acid functionality in a protein, such as amino group of lysine residues or the N-terminal amino group. Thiol specific functionality in iodoacetyl and maleimide reagents may react with amine groups, but higher pH (>9.0) and longer reaction times are required (Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London).

Cysteine engineered antibodies may be useful in the treatment of cancer and include antibodies specific for cell surface and transmembrane receptors, and tumor-associated antigens (TAA). Such antibodies may be used as naked antibodies (unconjugated to a drug or label moiety) or as antibody-drug conjugates (ADC). Cysteine engineered antibodies of the invention may be site-specifically and efficiently coupled with a thiol-reactive reagent. The thiol-reactive reagent may be a multifunctional linker reagent, a capture label reagent, a fluorophore reagent, or a drug-linker intermediate. The cysteine engineered antibody may be labeled with a detectable label, immobilized on a solid phase support and/or conjugated with a drug moiety. Thiol reactivity may be generalized to any antibody where substitution of amino acids with reactive cysteine amino acids may be made within the ranges in the light chain selected from amino acid ranges: L-10 to L-20; L-38 to L-48; L-105 to L-115; L-139 to L-149; L-163 to L-173; and within the ranges in the heavy chain selected from amino acid ranges: H-35 to H-45; H-83 to H-93; H-114 to H-127; and H-170 to H-184, and in the Fc region within the ranges selected from H-268 to H-291; H-319 to H-344; H-370 to H-380; and H-395 to H-405, where the numbering of amino acid positions begins at position 1 of the Kabat numbering system (Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and continues sequentially thereafter as disclosed in WO2006034488. Thiol reactivity may also be generalized to certain domains of an antibody, such as the light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. Cysteine replacements resulting in thiol reactivity values of 0.6 and higher may be made in the heavy chain constant domains α, δ, ε, γ, and μ of intact antibodies: IgA, IgD, IgE, IgG, and IgM, respectively, including the IgG subclasses: IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. Such antibodies and their uses are disclosed in WO2006/034488.

Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, transmembrane proteins, signalling proteins, cell survival regulatory factors, cell proliferation regulatory factors, molecules associated with (for e.g., known or suspected to contribute functionally to) tissue development or differentiation,

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lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in vasculogenesis and molecules associated with (for e.g., known or suspected to contribute functionally to) angiogenesis.

An antibody of the invention may be conjugated to other thiol-reactive agents in which the reactive group is, for example, a maleimide, an iodoacetamide, a pyridyl disulfide, or other thiol-reactive conjugation partner (Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Hermanson, G. in Bioconjugate Techniques (1996) Academic Press, San Diego, pp. 40-55, 643-671). The partner may be a cytotoxic agent (e.g. a toxin such as doxorubicin or pertussis toxin), a fluorophore such as a fluorescent dye like fluorescein or rhodamine, a chelating agent for an imaging or radiotherapeutic metal, a peptidyl or non-peptidyl label or detection tag, or a clearance-modifying agent such as various isomers of polyethylene glycol, a peptide that binds to a third component, or another carbohydrate or lipophilic agent.

In one aspect, antibodies of the invention may be conjugated with any label moiety which can be covalently attached to the antibody through a reactive moiety, an activated moiety, or a reactive cysteine thiol group (Singh et al (2002) Anal. Biochem. 304:147-15; Harlow E. and Lane, D. (1999) Using Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press, Cold Spring Harbor, NY; Lundblad R.L. (1991) Chemical Reagents for Protein Modification, 2nd ed. CRC Press, Boca Raton, FL). The attached label may function to: (i) provide a detectable signal; (ii) interact with a second label to modify the detectable signal provided by the first or second label, e.g. to give FRET (fluorescence resonance energy transfer); (iii) stabilize interactions or increase affinity of binding, with antigen or ligand; (iv) affect mobility, e.g. electrophoretic mobility or cell-permeability, by charge, hydrophobicity, shape, or other physical parameters, or (v) provide a capture moiety, to modulate ligand affinity, antibody/antigen binding, or ionic complexation.

Labelled cysteine engineered antibodies may be useful in diagnostic assays, e.g., for detecting expression of an antigen of interest in specific cells, tissues, or serum. For diagnostic applications, the antibody will typically be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

Radioisotopes (radionuclides), such as ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Radioisotope labelled antibodies are useful in receptor targeted imaging experiments. The antibody can be labeled with ligand reagents that bind, chelate or otherwise complex a radioisotope metal where the reagent is reactive with the engineered cysteine thiol of the

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antibody, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, NY, Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, TX). Radionuclides can be targetted via complexation with the antibody-drug conjugates of the invention (Wu et al (2005) Nature Biotechnology 23(9):1137-1146).

Linker reagents such as DOTA-maleimide (4-maleimidobutyramidobenzyl-DOTA) can be prepared by the reaction of aminobenzyl-DOTA with 4-maleimidobutyric acid (Fluka) activated with isopropylchloroformate (Aldrich), following the procedure of Axworthy et al (2000) Proc. Natl. Acad. Sci. USA 97(4):1802-1807). DOTA-maleimide reagents react with the free cysteine amino acids of the cysteine engineered antibodies and provide a metal complexing ligand on the antibody (Lewis et al (1998) Bioconj. Chem. 9:72-86). Chelating linker labelling reagents such as DOTA-NHS (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono (N-hydroxysuccinimide ester) are commercially available (Macrocyclics, Dallas, TX). Receptor target imaging with radionuclide labelled antibodies can provide a marker of pathway activation by detection and quantitation of progressive accumulation of antibodies in tumor tissue (Albert et al (1998) Bioorg. Med. Chem. Lett. 8:1207-1210). The conjugated radio-metals may remain intracellular following lysosomal degradation.

Metal-chelate complexes suitable as antibody labels for imaging experiments are disclosed: US 5342606; US 5428155; US 5316757; US 5480990; US 5462725; US 5428139; US 5385893; US 5739294; US 5750660; US 5834456; Hnatowich et al (1983) J. Immunol. Methods 65:147-157; Meares et al (1984) Anal. Biochem. 142:68-78; Mirzadeh et al (1990) Bioconjugate Chem. 1:59-65; Meares et al (1990) J. Cancer1990, Suppl. 10:21-26; Izard et al (1992) Bioconjugate Chem. 3:346-350; Nikula et al (1995) Nucl. Med. Biol. 22:387-90; Camera et al (1993) Nucl. Med. Biol. 20:955-62; Kukis et al (1998) J. Nucl. Med. 39:2105-2110; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Camera et al (1994) J. Nucl. Med. 21:640-646; Ruegg et al (1990) Cancer Res. 50:4221-4226; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Lee et al (2001) Cancer Res. 61:4474-4482; Mitchell, et al (2003) J. Nucl. Med. 44:1105-1112; Kobayashi et al (1999) Bioconjugate Chem. 10:103-111; Miederer et al (2004) J. Nucl. Med. 45:129-137; DeNardo et al (1998) Clinical Cancer Research 4:2483-90; Blend et al (2003) Cancer Biotherapy & Radiopharmaceuticals 18:355-363; Nikula et al (1999) J. Nucl. Med. 40:166-76; Kobayashi et al (1998) J. Nucl. Med. 39:829-36; Mardirossian et al (1993) Nucl. Med. Biol. 20:65-74; Roselli et al (1999) Cancer Biotherapy & Radiopharmaceuticals, 14:209-20.

(b) Fluorescent labels such as rare earth chelates (europium chelates), fluorescein types including FITC, 5-carboxyfluorescein, 6-carboxy fluorescein; rhodamine types including TAMRA; dansyl;

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Lissamine; cyanines; phycoerythrins; Texas Red; and analogs thereof. The fluorescent labels can be conjugated to antibodies using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescent dyes and fluorescent label reagents include those which are commercially available from Invitrogen/Molecular Probes (Eugene, OR) and Pierce Biotechnology, Inc. (Rockford, IL).

(c) Various enzyme-substrate labels are available or disclosed (US 4275149). The enzyme generally catalyzes a chemical alteration of a chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; US 4737456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al (1981) "Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay", in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic Press, New York, 73:147-166.

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRP) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review, see US 4275149 and US 4318980.

A label may be indirectly conjugated with an amino acid side chain, an acitivated amino acid side chain, a cysteine engineered antibody, and the like. For example, the antibody can be conjugated with

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biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin or streptavidin, or vice versa. Biotin binds selectively to streptavidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the polypeptide variant, the polypeptide variant is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten polypeptide variant (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the polypeptide variant can be achieved (Hermanson, G. (1996) in Bioconjugate Techniques Academic Press, San Diego).

The antibody of the present invention may be employed in any known assay method, such as ELISA, competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Zola, (1987) Monoclonal Antibodies: A Manual of Techniques, pp.147-158, CRC Press, Inc.).

A detection label may be useful for localizing, visualizing, and quantitating a binding or recognition event. The labelled antibodies of the invention can detect cell-surface receptors. Another use for detectably labelled antibodies is a method of bead-based immunocapture comprising conjugating a bead with a fluorescent labelled antibody and detecting a fluorescence signal upon binding of a ligand. Similar binding detection methodologies utilize the surface plasmon resonance (SPR) effect to measure and detect antibody-antigen interactions.

Detection labels such as fluorescent dyes and chemiluminescent dyes (Briggs et al (1997) "Synthesis of Functionalised Fluorescent Dyes and Their Coupling to Amines and Amino Acids," J. Chem. Soc., Perkin-Trans. 1:1051-1058) provide a detectable signal and are generally applicable for labelling antibodies, preferably with the following properties: (i) the labelled antibody should produce a very high signal with low background so that small quantities of antibodies can be sensitively detected in both cell-free and cell-based assays; and (ii) the labelled antibody should be photostable so that the fluorescent signal may be observed, monitored and recorded without significant photo bleaching. For applications involving cell surface binding of labelled antibody to membranes or cell surfaces, especially live cells, the labels preferably (iii) have good water-solubility to achieve effective conjugate concentration and detection sensitivity and (iv) are non-toxic to living cells so as not to disrupt the normal metabolic processes of the cells or cause premature cell death.

Direct quantification of cellular fluorescence intensity and enumeration of fluorescently labelled events, e.g. cell surface binding of peptide-dye conjugates may be conducted on an system (FMAT® 8100 HTS System, Applied Biosystems, Foster City, Calif.) that automates mix-and-read, non-radioactive assays with live cells or beads (Miraglia, "Homogeneous cell- and bead-based assays for high throughput screening using fluorometric microvolume assay technology", (1999) J. of Biomolecular Screening 4:193-

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204). Uses of labelled antibodies also include cell surface receptor binding assays, inmmunocapture assays, fluorescence linked immunosorbent assays (FLISA), caspase-cleavage (Zheng, "Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo", (1998) Proc. Natl. Acad. Sci. USA 95:618-23; US 6372907), apoptosis (Vermes, "A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V" (1995) J. Immunol. Methods 184:39-51) and cytotoxicity assays. Fluorometric microvolume assay technology can be used to identify the up or down regulation by a molecule that is targeted to the cell surface (Swartzman, "A homogeneous and multiplexed immunoassay for high-throughput screening using fluorometric microvolume assay technology", (1999) Anal. Biochem. 271:143-51).

Labelled antibodies of the invention are useful as imaging biomarkers and probes by the various methods and techniques of biomedical and molecular imaging such as: (i) MRI (magnetic resonance imaging); (ii) MicroCT (computerized tomography); (iii) SPECT (single photon emission computed tomography); (iv) PET (positron emission tomography) Chen et al (2004) Bioconjugate Chem. 15:41-49; (v) bioluminescence; (vi) fluorescence; and (vii) ultrasound. Immunoscintigraphy is an imaging procedure in which antibodies labeled with radioactive substances are administered to an animal or human patient and a picture is taken of sites in the body where the antibody localizes (US 6528624). Imaging biomarkers may be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers may be of several types: Type 0 are natural history markers of a disease and correlate longitudinally with known clinical indices, e.g. MRI assessment of synovial inflammation in rheumatoid arthritis; Type I markers capture the effect of an intervention in accordance with a mechanism-of-action, even though the mechanism may not be associated with clinical outcome; Type II markers function as surrogate endpoints where the change in, or signal from, the biomarker predicts a clinical benefit to "validate" the targeted response, such as measured bone erosion in rheumatoid arthritis by CT. Imaging biomarkers thus can provide pharmacodynamic (PD) therapeutic information about: (i) expression of a target protein, (ii) binding of a therapeutic to the target protein, i.e. selectivity, and (iii) clearance and half-life pharmacokinetic data. Advantages of in vivo imaging biomarkers relative to lab-based biomarkers include: non-invasive treatment, quantifiable, whole body assessment, repetitive dosing and assessment, i.e. multiple time points, and potentially transferable effects from preclinical (small animal) to clinical (human) results. For some applications, bioimaging supplants or minimizes the number of animal experiments in preclinical studies.

Peptide labelling methods are well known. See Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, (1997) Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means

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(1990) Bioconjugate Chem. 1:2; Glazer et al (1975) Chemical Modification of Proteins. Laboratory Techniques in Biochemistry and Molecular Biology (T. S. Work and E. Work, Eds.) American Elsevier Publishing Co., New York; Lundblad, R. L. and Noyes, C. M. (1984) Chemical Reagents for Protein Modification, Vols. I and II, CRC Press, New York; Pfleiderer, G. (1985) "Chemical Modification of Proteins", Modern Methods in Protein Chemistry, H. Tschesche, Ed., Walter DeGryter, Berlin and New York; and Wong (1991) Chemistry of Protein Conjugation and Cross-linking, CRC Press, Boca Raton, Fla.); De Leon-Rodriguez et al (2004) Chem.Eur. J. 10:1149-1155; Lewis et al (2001) Bioconjugate Chem. 12:320-324; Li et al (2002) Bioconjugate Chem. 13:110-115; Mier et al (2005) Bioconjugate Chem. 16:240-237.

Peptides and proteins labelled with two moieties, a fluorescent reporter and quencher in sufficient proximity undergo fluorescence resonance energy transfer (FRET). Reporter groups are typically fluorescent dyes that are excited by light at a certain wavelength and transfer energy to an acceptor, or quencher, group, with the appropriate Stokes shift for emission at maximal brightness. Fluorescent dyes include molecules with extended aromaticity, such as fluorescein and rhodamine, and their derivatives. The fluorescent reporter may be partially or significantly quenched by the quencher moiety in an intact peptide. Upon cleavage of the peptide by a peptidase or protease, a detectable increase in fluorescence may be measured (Knight, C. (1995) "Fluorimetric Assays of Proteolytic Enzymes", Methods in Enzymology, Academic Press, 248:18-34).

The labelled antibodies of the invention may also be used as an affinity purification agent. In this process, the labelled antibody is immobilized on a solid phase such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing the antigen to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the antigen to be purified, which is bound to the immobilized polypeptide variant. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the antigen from the polypeptide variant.

Labelling reagents typically bear reactive functionality which may react (i) directly with a cysteine thiol of a cysteine engineered antibody to form the labelled antibody, (ii) with a linker reagent to form a linker-label intermediate, or (iii) with a linker antibody to form the labelled antibody. Reactive functionality of labelling reagents include: maleimide, haloacetyl, iodoacetamide succinimidyl ester (e.g. NHS, N-hydroxysuccinimide), isothiocyanate, sulfonyl chloride, 2,6-dichlorotriazinyl, pentafluorophenyl ester, and phosphoramidite, although other functional groups can also be used.

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An exemplary reactive functional group is N-hydroxysuccinimidyl ester (NHS) of a carboxyl group substituent of a detectable label, e.g. biotin or a fluorescent dye. The NHS ester of the label may be preformed, isolated, purified, and/or characterized, or it may be formed in situ and reacted with a nucleophilic group of an antibody. Typically, the carboxyl form of the label is activated by reacting with some combination of a carbodiimide reagent, e.g. dicyclohexylcarbodiimide, diisopropylcarbodiimide, or a uronium reagent, e.g. TSTU (O-(N-Succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, HBTU (O-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), or HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), an activator, such as 1-hydroxybenzotriazole (HOBt), and N-hydroxysuccinimide to give the NHS ester of the label. In some cases, the label and the antibody may be coupled by in situ activation of the label and reaction with the antibody to form the label-antibody conjugate in one step. Other activating and coupling reagents include TBTU (2-(1H-benzotriazo-1-yl)-1-1,3,3-tetramethyluronium hexafluorophosphate), TFFH (N,N',N",N'"-tetramethyluronium 2-fluoro-hexafluorophosphate), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline), DCC (dicyclohexylcarbodiimide); DIPCDI (diisopropylcarbodiimide), MSNT (1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole, and aryl sulfonyl halides, e.g. triisopropylbenzenesulfonyl chloride.

Albumin binding peptide-Fab compounds of the invention:

In one aspect, the antibody of the invention is fused to an albumin binding protein. Plasma-protein binding can be an effective means of improving the pharmacokinetic properties of short lived molecules. Albumin is the most abundant protein in plasma. Serum albumin binding peptides (ABP) can alter the pharmacodynamics of fused active domain proteins, including alteration of tissue uptake, penetration, and diffusion. These pharmacodynamic parameters can be modulated by specific selection of the appropriate serum albumin binding peptide sequence (US 20040001827). A series of albumin binding peptides were identified by phage display screening (Dennis et al. (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" J Biol Chem. 277:35035-35043; WO 01/45746). Compounds of the invention include ABP sequences taught by: (i) Dennis et al (2002) J Biol Chem. 277:35035-35043 at Tables III and IV, page 35038; (ii) US 20040001827 at [0076] SEQ ID NOS: 9-22; and (iii) WO 01/45746 at pages 12-13, all of which are incorporated herein by reference. Albumin Binding (ABP)-Fabs are engineered by fusing an albumin binding peptide to, for example, the C-terminus of Fab heavy chain in 1:1 stoichiometric ratio (1 ABP / 1 Fab). It was shown that association of these ABP-Fabs with albumin increased antibody half life by more than 25 fold in rabbits and mice. The above described reactive Cys residues can therefore be introduced in these ABP-Fabs and used for site-specific conjugation with cytotoxic drugs followed by in vivo animal studies.

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Exemplary albumin binding peptide sequences include, but are not limited to, the amino acid sequences listed in SEQ ID NOS:80-84.

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	SEQ ID NO:80
QRLMEDICLPRWGCLWEDDF	SEQ ID NO:81
QRLIEDICLPRWGCLWEDDF	SEQ ID NO:82
RLIEDICLPRWGCLWEDD	SEQ ID NO:83
DICLPRWGCLW	SEQ ID NO:84

Antibody-Drug Conjugates

In another aspect, the invention provides immunoconjugates, or antibody-drug conjugates (ADC), comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). In another aspect, the invention further provides methods of using the immunoconjugates. In one aspect, an immunoconjugate comprises any of the above anti-STEAP-1 antibodies covalently attached to a cytotoxic agent or a detectable agent.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4,975,278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., (1986) supra). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) Jour. of the 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

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calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas) and cAC10 (specific to CD30 on hematological malignancies) (Doronina et al (2003) Nature Biotechnology 21(7):778-784) and are under therapeutic development.

Chemotherapeutic agents useful in the generation of immunoconjugates are described herein. Enzymatically active toxins and fragments thereof that 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, e.g., WO 93/21232 published October 28, 1993. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as

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N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al (1987) Science, 238:1098. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (WO94/11026).

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

Maytansine and maytansinoids

In some embodiments, the immunoconjugate comprises an antibody (full length or fragments) of the invention conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

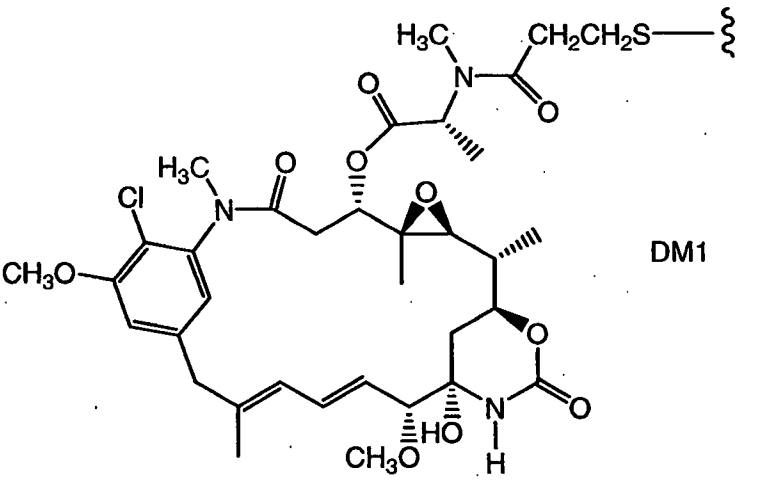
Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods.

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Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (US 4256746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (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

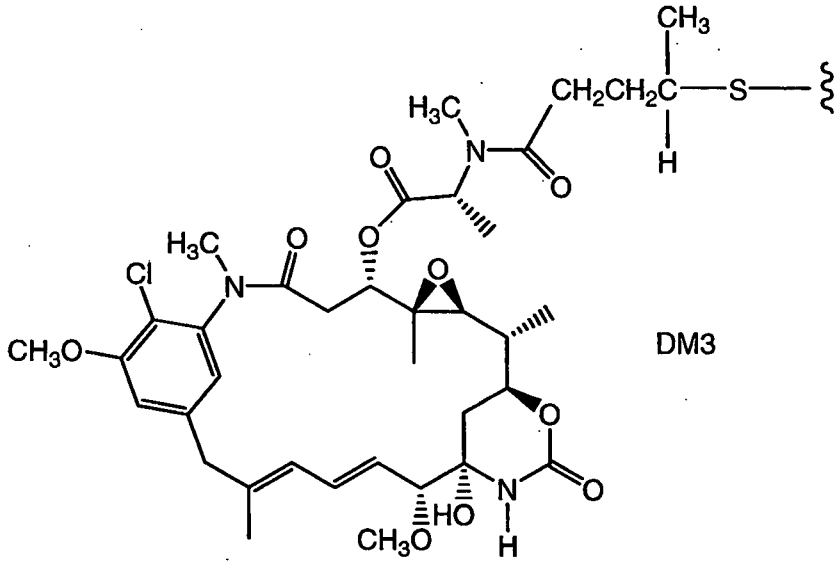
Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (US 4424219) (prepared by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (US 4450254) (prepared from Nocardia); C-15-hydroxy/acyloxy (US 4364866) (prepared by the conversion of maytansinol by Streptomyces); C-15-methoxy (US Pat. Nos. 4313946 and 4315929) (isolated from Trewia nudlflora); C-18-N-demethyl (US Pat. Nos. 4362663 and 4322348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (US 4371533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, having the structures:

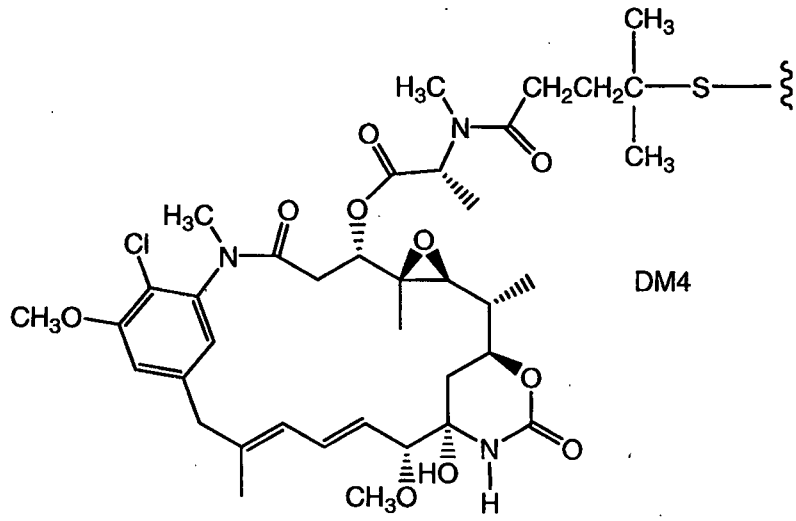


DM1

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DM3

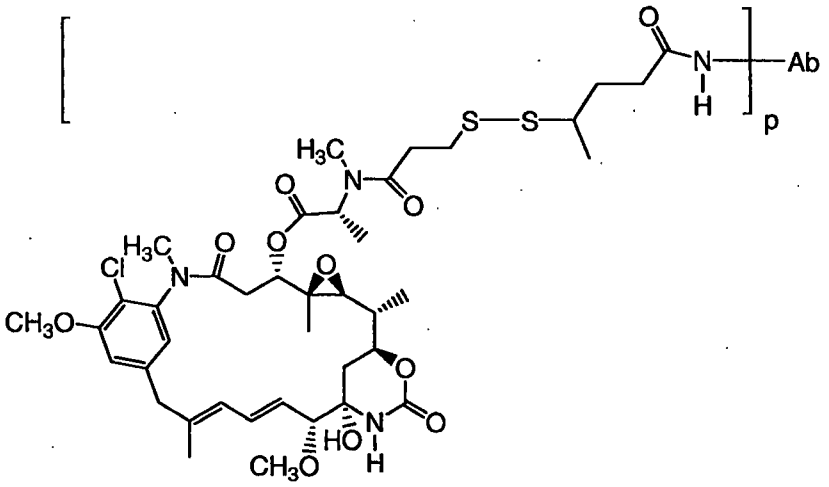


DM4

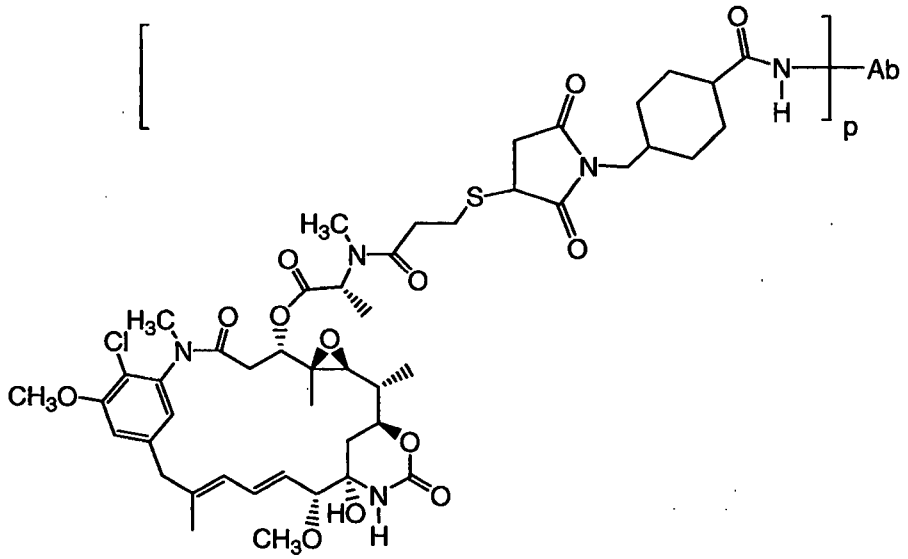
wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody drug conjugate. HERCEPTIN® (trastuzumab) linked by SMCC to DM1 has been reported (WO 2005/037992, which is expressly incorporated herein by reference in its entirety). An antibody drug conjugate of the present invention may be prepared according to the procedures disclosed therein.

Other exemplary maytansinoid antibody drug conjugates have the following structures and abbreviations, (wherein Ab is antibody and p is 1 to about 8):

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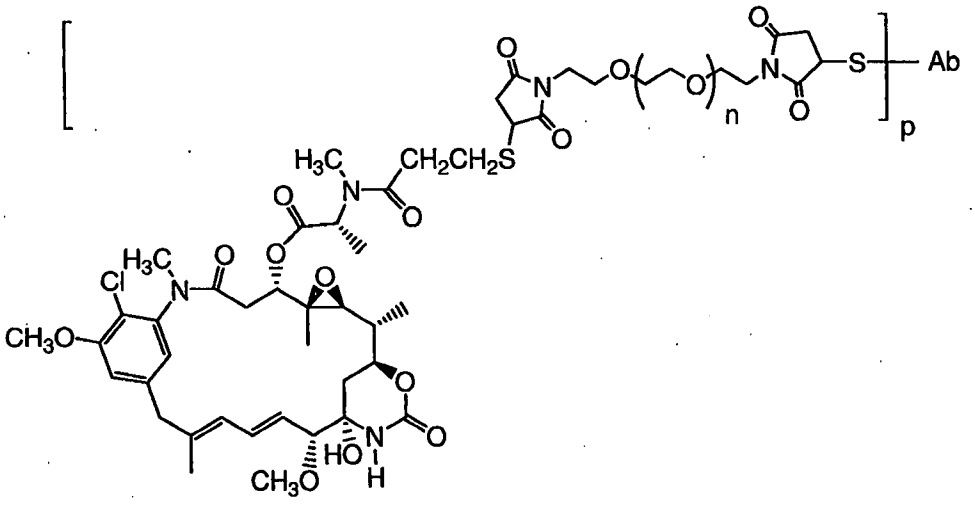
Ab -SPP-DM1



Ab-SMCC-DM1

Exemplary antibody drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:

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where Ab is antibody; n is 0, 1, or 2; and p is 1, 2, 3, or 4.

Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020; 5,416,064; 6,441163 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 immunoconjugates 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. Chari et al., Cancer Research 52:127-131 (1992) describe immunoconjugates 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 3 x 10^5 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.

Anti-STEAP-1 antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed,

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for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent Nos. 5208020, 6441163, or EP Patent 0 425 235 B1, Chari et al., Cancer Research 52:127-131 (1992), and US 2005/0169933 A1, the disclosures of which are hereby expressly incorporated by reference. Antibody-maytansinoid conjugates comprising the linker component SMCC may be prepared as disclosed in U.S. Patent Application No. 11/141344, filed 31 May 2005, "Antibody Drug Conjugates and Methods". The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents. Additional linking groups are described and exemplified herein.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 (1978)) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

In one embodiment, any of the antibodies of the invention (full length or fragment) is conjugated to one or more maytansinoid molecules. In one embodiment of the immunoconjugate, the cytotoxic agent D, is a maytansinoid DM1. In one embodiment of the immunoconjugate, the linker is selected from the group consisting of SPDP, SMCC, IT, SPDP, and SPP.

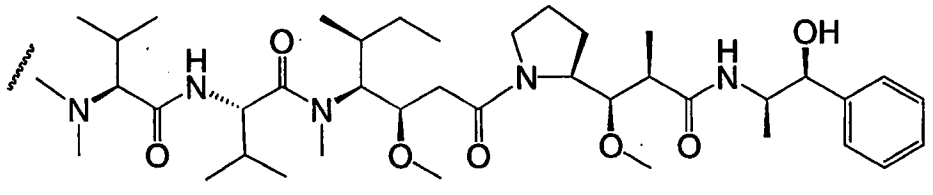
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Auristatins and dolostatins

In some embodiments, the immunoconjugate comprises an antibody of the invention conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (US Patent Nos. 5635483; 5780588). 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 (US 5663149) 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).

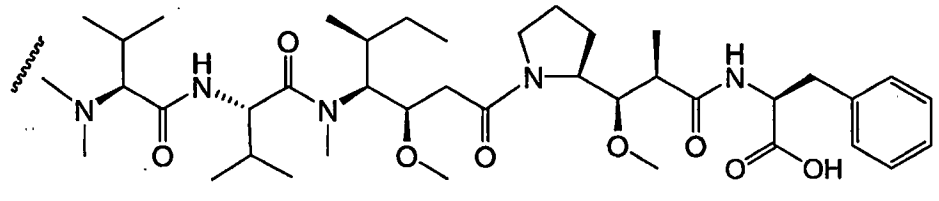
Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

An exemplary auristatin embodiment is MMAE (wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate).



MMAE

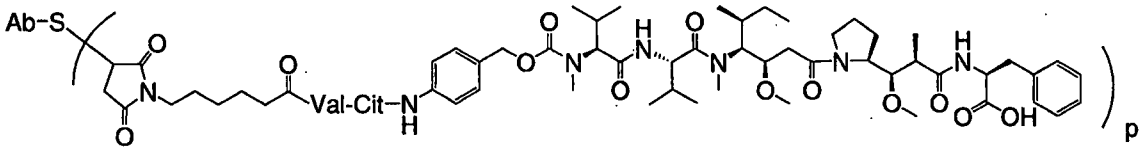
Another exemplary auristatin embodiment is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate (US 2005/0238649):



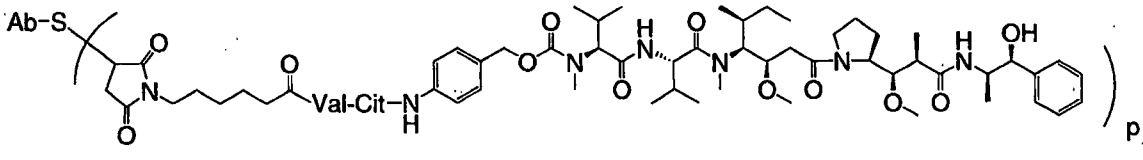
MMAF

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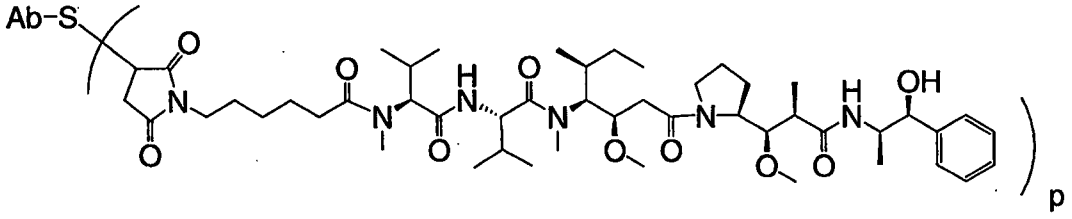
Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):



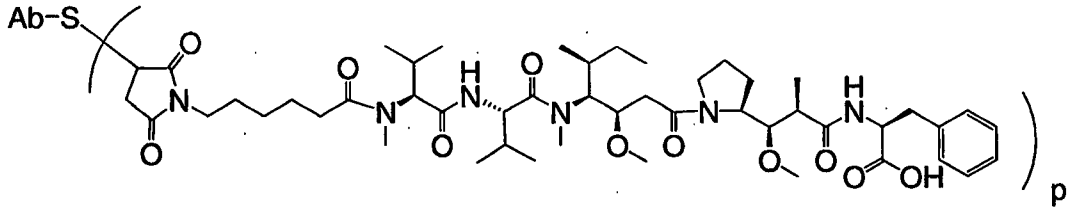
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

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. Schröder and K. Lübke, "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: US 5635483; US 5780588; 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.

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Calicheamicin

In other embodiments, the immunoconjugate comprises an antibody of the invention conjugated to one or more calicheamicin molecules. 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. patents 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, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman et al., Cancer Research 53:3336-3342 (1993), 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.

Other cytotoxic agents

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. patents 5,053,394, 5,770,710, as well as esperamicins (U.S. patent 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

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

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 At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic

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resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

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 tc^{99m} or I^{123}, .Re^{186}, Re^{188} and In^{111} 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.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

Preparation of antibody drug conjugates:

In the antibody drug conjugates (ADC) of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), e.g. about 1 to about 20 drug moieties per antibody, through a linker (L). In one

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embodiment, the number of drug moieties (D) per antibody is from about 1 to about 5, alternatively, from about 2 to about 6, alternatively, from about 2 to about 5, alternatively from about 3 to about 4 drug moieties per antibody. Because the number of drug moieties per antibody is typically an average number over all conjugates in a population of an antibody drug conjugate, the number of drug moieties per antibody may not be a whole number. The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent, to form Ab-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of an antibody. Additional methods for preparing ADC are described herein.

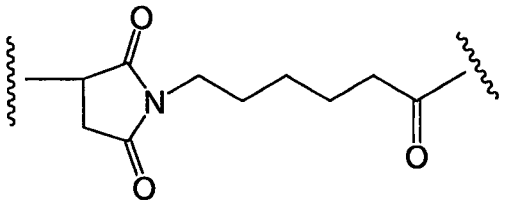
$$Ab–(L–D)_p \qquad \text{Formula I}$$

The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). In on embodiment, the linker is valine-citrullin-p-aminobenzyloxycaronyl ("vc-PAB"). Additional linker components are known in the art and some are described herein.

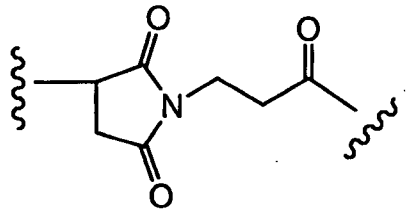
In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Exemplary linker component structures are shown below (wherein the wavy line indicates sites of covalent attachment to other components of the ADC):

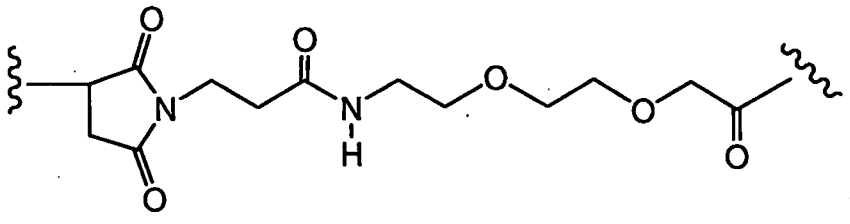
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MC

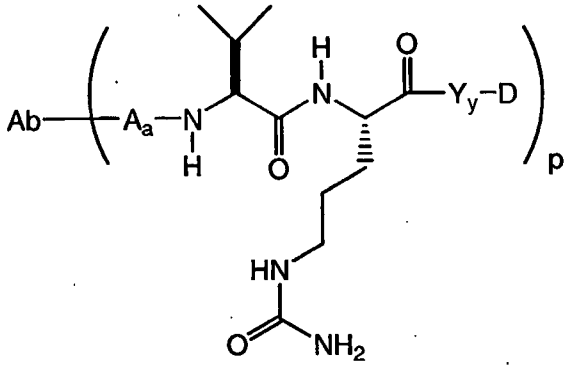


MP

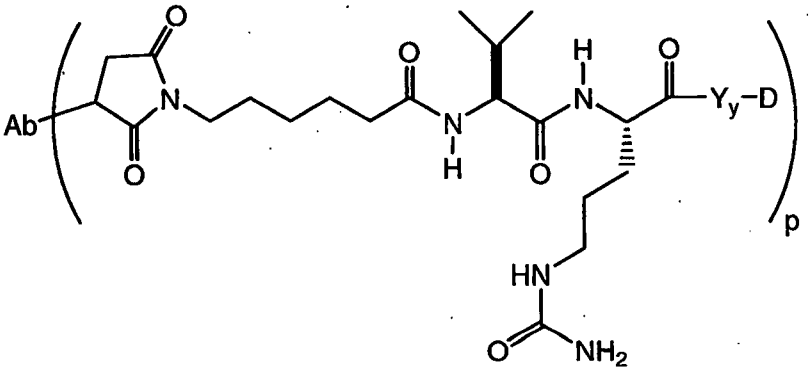


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Additional exemplary linker components and abbreviations include (wherein the antibody (Ab) and linker are depicted, and p is 1 to about 8):

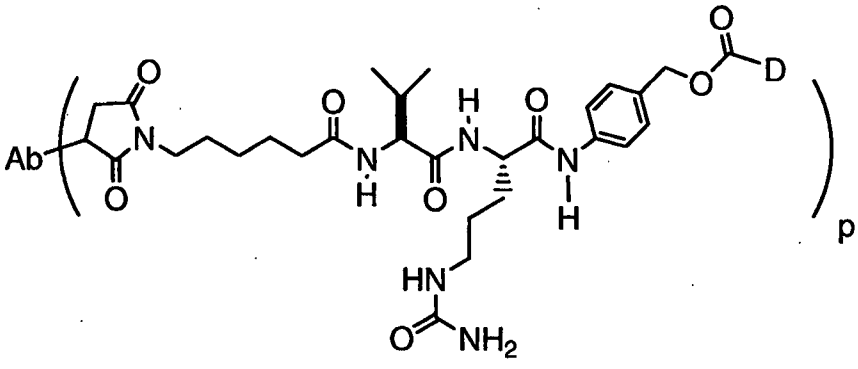


Val-cit



MC-val-cit

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MC-val-cit-PAB

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by introducing one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody drug conjugates of the invention may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic subsituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either glactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first

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amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such aldehyde can be reacted with a drug moiety or linker nucleophile.

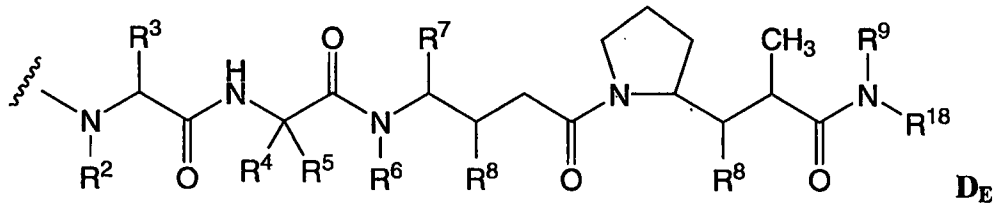
Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Methods for the conjugation of linker-drug moieties to cell-targeted proteins such as antibodies, immunoglobulins or fragments thereof are found, for example, in US5,208,020; US6,441,163; WO2005037992; WO2005081711; and WO2006/034488, all of which are hereby expressly incorporated by reference in their entirety.

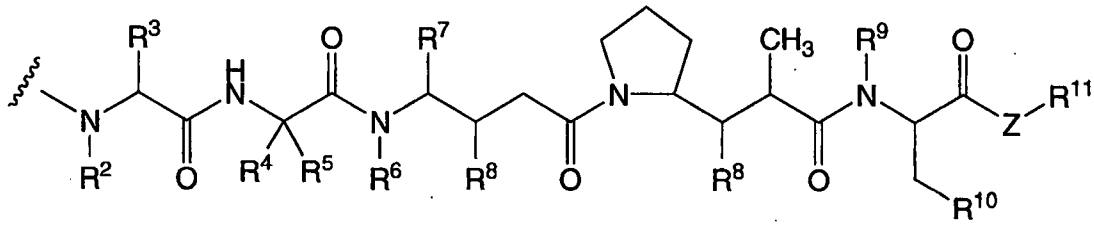
Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

In one embodiment of the immunoconjugate, the cytotoxic agent, D, is an auristatin of formula D_E or D_F



D_E



D_F

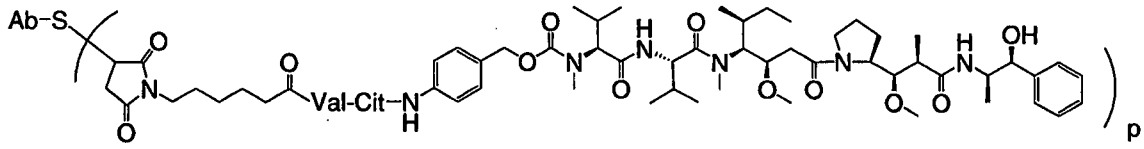
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and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $–(CH_2)_2–O–(CH_2)_2–O–(CH_2)_2–O–CH_3$; and R^{18} is $–C(R^8)_2–C(R^8)_2–$aryl; and

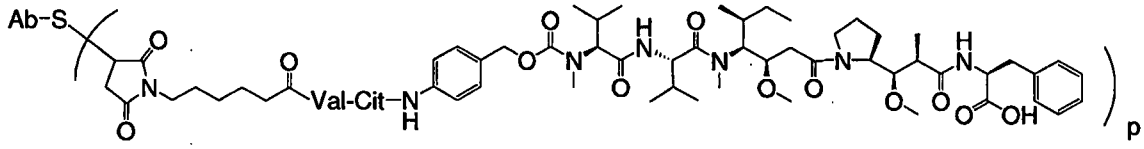
(d) p ranges from about 1 to 8.

The following embodiments are further provided for any of the above immunoconjugates. In one embodiment, an immunoconjugate has in vitro or in vivo cell killing activity. In one embodiment, the linker is attached to the antibody through a thiol group on the antibody. In one embodiment, the linker is cleavable by a protease. In one embodiment, the linker comprises a val-cit dipeptide. In one embodiment, the linker comprises a p-aminobenzyl unit. In one embodiment, the p-aminobenzyl unit is disposed between the drug and a protease cleavage site in the linker. In one embodiment, the p-aminobenzyl unit is p-aminobenzyloxycarbonyl (PAB). In one embodiment, the linker comprises 6-maleimidocaproyl. In one embodiment, the 6-maleimidocaproyl is disposed between the antibody and a protease cleavage site in the linker. The above embodiments may occur singly or in any combination with one another.

In one embodiment, the drug is selected from MMAE and MMAF. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 2 to about 5. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 1 to about 6, from about 2 to about 5, from about 2 to about 6, from about 2 to about 4, from about 2 to about 3, from about 3 to about 4, from about 3 to about 5, from about 3 to about 6, or from about 4 to about 6.

Labelled antibody imaging methods:

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In another embodiment of the invention, cysteine engineered antibodies may be labelled through the cysteine thiol with radionuclides, fluorescent dyes, bioluminescence-triggering substrate moieties, chemiluminescence-triggering substrate moieties, enzymes, and other detection labels for imaging experiments with diagnostic, pharmacodynamic, and therapeutic applications. Generally, the labelled cysteine engineered antibody, i.e. "biomarker" or "probe", is administered by injection, perfusion, or oral ingestion to a living organism, e.g. human, rodent, or other small animal, a perfused organ, or tissue sample. The distribution of the probe is detected over a time course and represented by an image.

Articles of Manufacture:

In another embodiment of the invention, an article of manufacture, or "kit", containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds an antibody-drug conjugate (ADC) composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an ADC. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Pharmaceutical compositions:

In one aspect, a pharmaceutical composition is provided comprising any of the above immunoconjugates and a pharmaceutically acceptable carrier. In one aspect, a method of treating a prostate, lung, colon, bladder, or ovarian cell proliferative disorder, or Ewing's sarcoma is provided, wherein the method comprises administering to an individual the pharmaceutical composition. In one embodiment, the prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma cell proliferative disorder is a metastasis of a primary prostate, lung, colon, bladder, or ovarian cancer or Ewing's sarcoma. In one embodiment, the cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a cell.

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In one aspect, a method of inhibiting cell proliferation is provided, wherein the method comprises exposing a cell to any of the above immunoconjugates under conditions permissive for binding of the immunoconjugate to STEAP-1. In one embodiment, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma is a tumor cell. In one embodiment, the tumor cell is a prostate, lung, colon, bladder, or ovarian tumor cell or Ewing's sarcoma celll of a mammal experiencing or suspected of experiencing prostate, lung, colon, bladder cell or Ewing's sarcoma proliferative disorder including, but not limited to, a metastasis of a primary prostate, lung, colon, bladder cell cancer tumor or Ewing's sarcoma tumor. In one embodiment, the prostate, lung, colon, bladder cell or Ewing's sarcoma is a xenograft. In one embodiment, the exposing takes place in vitro. In one embodiment, the exposing takes place in vivo.

In one aspect, a method of using the anti-STEAP-1 antibody of the invention is provided to assay serum soluble STEAP-1 in a mammal experiencing prostate lung or colon cell proliferative disorder (or metastasis of a primary incidence of such disorder), measuring clinical progression or regression of the diseases, or assess tumor burden or relapse.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the amino acid sequence of human STEAP-1 (SEQ ID NO:1) aligned with STEAP-1 from mouse and cynomolgus monkey (cyno) (SEQ ID NOs:2 and 3, respectively). Extracellular domains 1, 2, and 3 are labeled and marked by shaded boxes.

Figures 2A-2B: **Figure 2A** depicts the amino acid sequence of the light chain variable region of murine 120.545 anti-STEAP-1 antibody aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human subgroup III sequence. The CDRs are boxed (CDR-L1, CDR-L2, and CDR-L3). The sequences bracketing the CDRs are the framework sequences (FR-L1 to FR-L4). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs. **Figure 2B** depicts the amino acid sequence of the heavy chain variable region of murine anti-STEAP-1 antibody (120.545) aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human kappa I sequence. Humanized variants 24, 37, 48, 67, and 37/48, 67, 71, and 78 were prepared by making the following amino acid changes: A24V, V37I, V48M, F67I, and L78F in the heavy chain of the 120 graft antibody. The CDRs are boxed. The FR-H1, FR-H2, FR-H3 and FR-H4 sequences bracket the CDRs (CDR-H1, CDR-H2, and CDR-H3). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs.

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Figures 3A and 3B show exemplary acceptor human variable heavy (VH) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows, where the FR SEQ ID NOs are listed in the order FR-H1, FR-H2, FR-H3, FR-H4:

- human VH subgroup I consensus framework "A" minus Kabat CDRs (SEQ ID NOs:26, 27, 28, 29).

- human VH subgroup I consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:30, 31, 28, 29; SEQ ID NOs:30, 31, 32, 29; and SEQ ID NOs:30, 31, 33, 29).

- human VH subgroup II consensus framework "A" minus Kabat CDRs (SEQ ID NOs:34, 35, 36, 29).

- human VH subgroup II consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:37, 38, 36, 29; SEQ ID NOs:37, 38, 39, 29; and SEQ ID NOs:37, 38, 40, 29).

- human VH subgroup III consensus framework "A" minus Kabat CDRs (SEQ ID NOs:41, 42, 43, 29).

- human VH subgroup III consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 43, 29; SEQ ID NOs:44, 45, 46, 29; and SEQ ID NOs:44, 45, 46, 29).

- human VH acceptor 1 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 49, 29).

- human VH acceptor frameworks "B" and "C" minus extended hypervariable regions (SEQ ID NOs:44, 45, 49, 29; and SEQ ID NOs:44, 45, 50, 29).

- human VH acceptor 2 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 51, 29).

- human VH acceptor 2 framework "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 51, 29; SEQ ID NOs:44, 45, 52, 29; and SEQ ID NOs:44, 45, 53, 29).

Figures 4A and 4B show exemplary acceptor human variable light (VL) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows:

- human VL kappa subgroup I-1 consensus framework (κv1-1): SEQ ID NOs:54, 55, 56, 57

- human VL kappa subgroup I consensus framework (κv1): SEQ ID NOs:54, 58, 56, 57

- human VL kappa subgroup II consensus framework (κv2): SEQ ID NOs:58, 59, 60, 57

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- human VL kappa subgroup III consensus framework (κv3): SEQ ID NOs:61, 62, 63, 57

- human VL kappa subgroup IV consensus framework (κv4): SEQ ID NOs:64, 65, 66, 57.

Figure 5 depicts alignments of native sequence human IgG Fc region sequences, humIgG1 (non-A allotype, SEQ ID NO:85; and A allotype, where the amino acid sequence SREEM within SEQ ID NO:85 is changed to SRDEL), humIgG2 (SEQ ID NO:86), humIgG3 (SEQ ID NO:87) and humIgG4 (SEQ ID NO:88) with differences between the sequences marked with asterisks. Numbers above the sequences represent the EU numbering system. An exemplary kappa constant region is also shown.

Figures 6A-6D depicts a FACS analysis normalized for the level of display of each antibody or variant on phage. Figure 6A shows FACS shifts for four exemplary antibodies. Figure 6B is an alignment of FACS shifts for several antibodies as indicated in the figure and in Example 1. Figures 6C and 6D are FACS shift alignments after normalization for phage display levels.

Figures 7A-7F graphically depict FACS analyses showing binding of anti-STEAP-1 that the anti-STEAP-1 murine, chimera and humanized version 24 antibodies to human STEAP-1 express on the cell surface. Figures 7A-7C indicate that anti-STEAP-1 murine 120, chimera 120 and humanized 120v.24 bind human and cynomolgus monkey STEAP-1, but not mouse STEAP-1. Figures 7D-7F are FACS plots showing binding of murine 120, 120 chimera, and humanized 120v.24 (clone 67) to human STEAP-1 expressed on the cell surface. Exogenous STEAP-1 was stably expressed in 293 cells (designated LB50 cells) and PC3 cells (designated PS5.4 cells) (Figures 7D and 7E), and endogenously expressed in LNCaP BR cells (Figure 7F).

Figures 8A and 8B. Figure 8A is a graph showing that administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. See Example 4. **Figure 8B** is a graph showing that single dose administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg), and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a LNCaP cell xenograft prostate tumor model. See Example 4.

Figure 9 is a graph showing that administration of anti-STEAP-1antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE) at 3 mg/kg, or anti-STEAP-1 120 chimera-MC-MMAF (abbreviated anti-STEAP mcMMAF) at 6 mg/kg, was shown to be efficacious in a prostate cancer xenograft model of castrated SCID-beige mice transplanted with LNCaP cells. See Example 4.

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Figure 10 is a graph showing that administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE (at 3 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. See Example 4.

Figure 11 is a graph showing that administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, humanized anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. See Example 4.

Figure 12 is a diagram depicting STEAP-1 embedded in a cell membrane. Anti-STEAP-1 antibody 120 binding is conformation dependent and does not recognize to a linear epitope of STEAP-1.

Figures 13A-13D show STEAP-1 expressed on the surface of cells as detected by immunohistochemistry. **Figure 13A** shows an immunohistochemical stain of 293 cells expressing exogenous STEAP-1 on the cell surface. **Figure 13B** shows an immunohistochemical stain of PC3 cells expressing exogenous STEAP-1 on the cell surface. **Figure 13C** shows an immunohistochemical stain of LNCaP cells expressing endogenous STEAP-1 on the cell surface. **Figure 13D** shows an immunohistochemica stain of LuCAP 77 cells expressing endogenous STEAP-1 on the cell surface.

Figures 14A-14E are graphs showing the relative effectiveness of anti-STEAP-1 antibody 120v.24-MCMMAF and anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE to kill STEAP-1 expressing cells in vitro. PS5.4 cells (**Figure 14A**) are PC3 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. LB50 cells (**Figure 14B**) are 293 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. LNCaP cells (**Figure 14C**) express STEAP-1 endogenously. "PC3 vec" (**Figure 14D**) and "293 vec" (**Figure 14E**) refer to 293 cells and PC3 cells, respectively, transformed with a vector control.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Isolated antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are further provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as a tumor or cancer. In certain embodiments, STEAP-1 is expressed in tumor or cancer of prostate, lung, or colon tissue. In certain

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embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1, e.g., STEAP-1 expressed on the cell surface. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1 expression on the surface of normal and/or tumor or cancer cells of protate, lung or colon tissue.

Polynucleotides encoding anti-STEAP-1 antibodies are provided. Vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided, and host cells comprising such vectors are provided. Compositions, including pharmaceutical formulations, comprising any one or more of the polynucleotides, anti-STEAP-1 antibodies, or immunoconjugates of the invention are also provided.

Methods of treating a cell proliferative disorder, including but not limited to tumor or cancer, with an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, treatment of tumor or cancer in prostate, lung or colon of a mammal. Methods of detecting STEAP-1 expression on a tissue cell using an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, detection of STEAP-1 expression on, as a non-limiting example, a normal cell, tumor cell, or cancer cell of prostate, lung, or colon cell.

General Techniques

The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds., (2003)); the series Methods in Enzymology (Academic Press, Inc.): Pcr 2: A Practical Approach (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture (R. I. Freshney, ed. (1987)); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney), ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D.

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Catty., ed., IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V. T. DeVita et al., eds., J.B. Lippincott Company, 1993).

DEFINITIONS AND ABBREVIATIONS

Definitions

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

An "isolated" nucleic acid molecule is a nucleic acid molecule that is separated from at least one other nucleic acid molecule with which it is ordinarily associated, for example, in its natural environment. An isolated nucleic acid molecule further includes a nucleic acid molecule contained in cells that ordinarily express the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Purified" means that a molecule is present in a sample at a concentration of at least 95% by weight, or at least 98% by weight of the sample in which it is contained.

The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values).

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The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors," or simply, "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of

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modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

"Oligonucleotide," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid

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sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "STEAP-1," as used herein, refers to any native STEAP-1 from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed STEAP-1 as well as any form of STEAP-1 that results from processing in the cell. The term also encompasses naturally occurring variants of STEAP-1, e.g., splice variants, allelic variants, and isoforms. The amino acid sequence of human STEAP-1 is depicted in Figure 1 (SEQ ID NO:1). In one embodiment, STEAP-1 is expressed on the cell surface, such as on the surface of a normal prostate, lung or colon cell, and has increased expression in prostate, lung or colon cancer cells or metastases of such cancer cells. Figure 1

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also depicts the amino acid sequence of STEAP-1 from mouse and cynomolgus monkey (SEQ ID NOs:2 and 3, respectively).

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having similar structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, monovalent antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized and/or affinity matured.

The term "anti-STEAP-1 antibody" or "an antibody that binds to STEAP-1" refers to an antibody that is capable of binding STEAP-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting STEAP-1. Preferably, the extent of binding of an anti-STEAP-1 antibody to an unrelated, non-STEAP-1 protein is less than about 10% of the binding of the antibody to STEAP-1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to STEAP-1 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an anti-STEAP-1 antibody binds to an epitope of STEAP-1 that is conserved among STEAP-1 from different species.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR).

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The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

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

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

"Antibody fragments" comprise only a portion of an intact antibody, wherein the portion retains at least one, and as many as most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half life substantially similar to an intact antibody. For example,

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such an antibody fragment may comprise on antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

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

"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL)

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in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO93/1161; Hudson et al. (2003) Nat. Med. 9:129-134; and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al. (2003) Nat. Med. 9:129-134.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler et al., Nature, 256: 495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage display technologies (see, e.g., Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and

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Lee et al., J. Immunol. Methods 284(1-2): 119-132(2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893; WO96/34096; WO96/33735; WO91/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks et al., Bio.Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996) and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

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A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six hypervariable regions, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. Xu et al. (2000) Immunity 13:37-45; Johnson and Wu (2003) in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. Hamers-Casterman et al. (1993) Nature 363:446-448; Sheriff et al. (1996) Nature Struct. Biol. 3:733-736.

A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact	
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L1	L24-L34	L24-L34	L26-L32	L30-L36	
L2	L50-L56	L50-L56	L50-L52	L46-L55	
L3	L89-L97	L89-L97	L91-L96	L89-L96	
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B	(Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35	(Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58	

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H3	H95-H102	H95-H102	H96-H101	H93-H101

Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions. The HVR-H1, HVR-H2, and HVR-H3 hypervariable regions of the humanized anti-STEAP-1 120v.24 antibodies of the invention are H26-H35A, H49-H6, and H95-H102 using Kabat numbering. The HVR-L1, HVR-L2, and HVR-L3 hypervariable regions of the humanized anti-STEP-1 120v.24 antibodies of the invention are L24-34, L50-56, and L89-97 using Kabat numbering. As used herein, the terms "HVR" and "CDR" are used interchangeably.

"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

An "affinity matured" antibody is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In one embodiment, an affinity matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. Bio/Technology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of HVR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

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A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds. Certain blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

An "agonist antibody," as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, e.g., Ghetie 1997, Hinton 2004). Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates administered with the Fc variant polypeptides.

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WO00/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. The content of that patent publication is specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source, e.g., from blood.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or Presta U.S. Patent No. 6,737,056 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

"Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed.

Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US Patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

The term "Fc region-comprising polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin, which comprises an Fc region. The C-terminal lysine (residue 447 according to the EU

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numbering system) of the Fc region may be removed, for example, during purification of the polypeptide or by recombinant engineering the nucleic acid encoding the polypeptide. Accordingly, a composition comprising a polypeptide having an Fc region according to this invention can comprise polypeptides with K447, with all K447 removed, or a mixture of polypeptides with and without the K447 residue.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferably those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may be 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat et al., supra. In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: EVQLVESGGGLVQPGGSLRLSCAAS (FR-H1, SEQ ID NO:21)-HVR-H1-WVRQAPGKGLEWV (FR-H2, SEQ ID NO:22)-HVR-H2-RFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR (FR-H3, SEQ ID NO:23)-HVR-H3-WGQGTLVTVSS (FR-H4, SEQ ID NO:24).

A "VL subgroup I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat et al., supra. In one embodiment, the VH subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the

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following sequences: DIQMTQSPSSLSASVGDRVTITC (FR-L1, SEQ ID NO:17)-HVR-L1-WYQQKPGKAPKLLIY (FR-L2, SEQ ID NO:18)-HVR-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, SEQ ID NO:19)-HVR-L3-FGQGTKVEIKR (FR-L4, SEQ ID NO:20). "Secretion signal sequence" or "signal sequence" refers to a nucleic acid sequence encoding a short signal peptide that can be used to direct a newly synthesized protein of interest through a cellular membrane, usually the inner membrane or both inner and outer membranes of prokaryotes. As such, the protein of interest such as the immunoglobulin light or heavy chain polypeptide is secreted into the periplasm of the prokaryotic host cells or into the culture medium. The signal peptide encoded by the secretion signal sequence may be endogenous to the host cells, or they may be exogenous, including signal peptides native to the polypeptide to be expressed. Secretion signal sequences are typically present at the amino terminus of a polypeptide to be expressed, and are typically removed enzymatically between biosynthesis and secretion of the polypeptide from the cytoplasm. Thus, the signal peptide is usually not present in a mature protein product.

"Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (125I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen, et al., (1999) J. Mol. Biol. 293:865-881). To establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the

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anti-VEGF antibody, Fab-12, in Presta et al., (1997) Cancer Res. 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates have dried, 150 µl/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcoreTM-2000 or a BIAcoreTM-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881. If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stirred cuvette.

An "on-rate," "rate of association," "association rate," or "k_{on}" according to this invention can also be determined as described above using a BIAcore™-2000 or a BIAcore™-3000 system (BIAcore®, Inc., Piscataway, NJ).

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A "disorder" is any condition or disease that would benefit from treatment with an substance/molecule or method of the invention. This includes chronic and acute disorders including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancerous conditions such as cancers or metastases of prostate, lung, and colon.

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

"Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain , as well as head and neck cancer, and associated metastases.

A "STEAP-1-expressing cell" is a cell which expresses endogenous or transfected STEAP-1 on the cell surface. A "STEAP-1-expressing cancer" is a cancer comprising cells that have STEAP-1 protein present on the cell surface. A "STEAP-1-expressing cancer" produces sufficient levels of STEAP-1 on the surface of cells thereof, such that an anti-STEAP-1 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which "overexpresses" STEAP-1 is one which has significantly higher levels of STEAP-1 at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by

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gene amplification or by increased transcription or translation. STEAP-1 overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the STEAP-1 protein present on the surface of a cell (e.g. via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of STEAP-1-encoding nucleic acid or mRNA in the cell, e.g. via fluorescent in situ hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study STEAP-1 overexpression by measuring shed antigen in a biological fluid such as serum, e.g, using antibody-based assays (see also, e.g., U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias et al. J. Immunol. Methods 132: 73-80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A STEAP-1-expressing cancer includes prostate, lung, and colon cancer.

As used herein, "treatment" (and variations such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). For prostate cancer, the progress of therapy can be assessed by routine methods, usually by measuring serum PSA (prostate specific antigen) levels; the higher the level of PSA in the blood, the more extensive the cancer. Commercial assays for detecting PSA are available, e.g, Hybitech Tandem-E and Tandem-R PSA assay kits, the Yang ProsCheck polyclonal assay (Yang Labs, Bellevue, WA), Abbott Imx (Abbott Labs, Abbott Park, IL), etc. Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can

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also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver, respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

An "individual" is a vertebrate. In certain embodiments, the vertebrate is a mammal. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, a mammal is a human.

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a substance/molecule of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids;

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antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Label" as used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-PSCA antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

"Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (*e.g.*, the ColE1 origin of replication) and a selectable marker (*e.g.*, ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic cells. Suitable vectors are disclosed below.

The cell that produces an anti-STEAP-1 antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a PSCA expressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be

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one which significantly reduces the percentage of PSCA expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu), chemotherapeutic agents (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, toxins, growth inhibitory agents, drug moieties, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "toxin" is any substance capable of having a detrimental effect on the growth or proliferation of a cell.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the

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synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine

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(NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROCAL® etidronate, NE-58095, ZOMETA® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell (such as a cell expressing STEAP-1) either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells (such as a cell expressing STEAP-1) in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a

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place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "intracellular metabolite" refers to a compound resulting from a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC). The metabolic process or reaction may be an enzymatic process, such as proteolytic cleavage of a peptide linker of the ADC, or hydrolysis of a functional group such as a hydrazone, ester, or amide. Intracellular metabolites include, but are not limited to, antibodies and free drug which have undergone intracellular cleavage after entry, diffusion, uptake or transport into a cell.

The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC) whereby the covalent attachment, i.e. linker, between the drug moiety (D) and the antibody (Ab) is broken, resulting in the free drug dissociated from the antibody inside the cell. The cleaved moieties of the ADC are thus intracellular metabolites.

The term "bioavailability" refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

The term "cytotoxic activity" refers to a cell-killing, cytostatic or growth inhibitory effect of an antibody-drug conjugate or an intracellular metabolite of an antibody-drug conjugate. Cytotoxic activity may be expressed as the IC_{50} value, which is the concentration (molar or mass) per unit volume at which half the cells survive.

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"Alkyl" is C1-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH3), ethyl (Et, -CH2CH3), 1-propyl (n-Pr, n-propyl, -CH2CH2CH3), 2-propyl (i-Pr, i-propyl, -CH(CH3)2), 1-butyl (n-Bu, n-butyl, -CH2CH2CH2CH3), 2-methyl-1-propyl (i-Bu, i-butyl, -CH2CH(CH3)2), 2-butyl (s-Bu, s-butyl, -CH(CH3)CH2CH3), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH3)3), 1-pentyl (n-pentyl, -CH2CH2CH2CH2CH3), 2-pentyl (-CH(CH3)CH2CH2CH3), 3-pentyl (-CH(CH2CH3)2), 2-methyl-2-butyl (-C(CH3)2CH2CH3), 3-methyl-2-butyl (-CH(CH3)CH(CH3)2), 3-methyl-1-butyl (-CH2CH2CH(CH3)2), 2-methyl-1-butyl (-CH2CH(CH3)CH2CH3), 1-hexyl (-CH2CH2CH2CH2CH2CH3), 2-hexyl (-CH(CH3)CH2CH2CH2CH3), 3-hexyl (-CH(CH2CH3)(CH2CH2CH3)), 2-methyl-2-pentyl (-C(CH3)2CH2CH2CH3), 3-methyl-2-pentyl (-CH(CH3)CH(CH3)CH2CH3), 4-methyl-2-pentyl (-CH(CH3)CH2CH(CH3)2), 3-methyl-3-pentyl (-C(CH3)(CH2CH3)2), 2-methyl-3-pentyl (-CH(CH2CH3)CH(CH3)2), 2,3-dimethyl-2-butyl (-C(CH3)2CH(CH3)2), 3,3-dimethyl-2-butyl (-CH(CH3)C(CH3)3.

The term "C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl,-acetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isoheptyl, n-octyl, and isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Alkenyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH_2), allyl (-CH_2CH=CH_2), cyclopentenyl (-C_5H_7), and 5-hexenyl (-CH_2 $CH_2CH_2CH_2$CH=CH_2)

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"Alkynyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic (-C≡CH) and propargyl (-CH_2C≡CH),

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH_2-) 1,2-ethyl (-CH_2CH_2-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

A "C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula -$(CH_2)_{1\text{-}10}$-. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

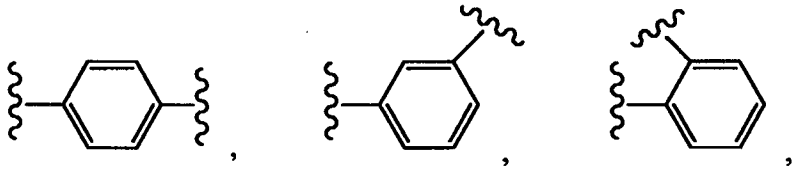
"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:

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in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

"Substituted alkyl," "substituted aryl," and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O^-, -OR, -SR, -S^-, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, NC(=O)R, -C(=O)R, -C(=O)NR_2, -SO_3^-, -SO_3H, -$S(=O)_2$R, -$OS(=O)_2$OR, -$S(=O)_2$NR, -S(=O)R, -$OP(=O)(OR)_2$, -$P(=O)(OR)_2$, -PO^-_3, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2^-, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR_2, -C(=S)NR_2, -C(=NR)NR_2, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C_2-C_{18} alkyl, C_6-C_{20} aryl, C_3-C_{14} heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

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"Heteroaryl" and "heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-

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pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

A "C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

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A "C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -$N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

A "C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York.

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Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

"Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

Abbreviations

LINKER COMPONENTS:

MC = 6-maleimidocaproyl

Val-Cit or "vc" = valine-citrulline (an exemplary dipeptide in a protease cleavable linker)

Citrulline = 2-amino-5-ureido pentanoic acid

PAB = p-aminobenzyloxycarbonyl (an example of a "self immolative" linker component)

Me-Val-Cit = N-methyl-valine-citrulline (wherein the linker peptide bond has been modified to prevent its cleavage by cathepsin B)

MC(PEG)6-OH = maleimidocaproyl- polyethylene glycol (can be attached to antibody cysteines).

SPP = N-succinimidyl-4-(2-pyridylthio)pentanoate

SPDP = N-succinimidyl-3-(2-pyridyldithio) propionate

SMCC = succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate

IT = iminothiolane

CYTOTOXIC DRUGS:

MMAE = mono-methyl auristatin E (MW 718)

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MMAF = variant of auristatin E (MMAE) with a phenylalanine at the C-terminus of the drug (MW 731.5)

MMAF-DMAEA = MMAF with DMAEA (dimethylaminoethylamine) in an amide linkage to the C-terminal phenylalanine (MW 801.5)

MMAF-TEG = MMAF with tetraethylene glycol esterified to the phenylalanine

MMAF-NtBu = N-t-butyl, attached as an amide to C-terminus of MMAF

DM1 = N(2')-deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine

DM3 = N(2')-deacetyl-N2-(4-mercapto-1-oxopentyl)-maytansine

DM4 = N(2')-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl)-maytansine

Further abbreviations are as follows: AE is auristatin E, Boc is N-(t-butoxycarbonyl), cit is citrulline, dap is dolaproine, DCC is 1,3-dicyclohexylcarbodiimide, DCM is dichloromethane, DEA is diethylamine, DEAD is diethylazodicarboxylate, DEPC is diethylphosphorylcyanidate, DIAD is diisopropylazodicarboxylate, DIEA is N,N-diisopropylethylamine, dil is dolaisoleucine, DMA is dimethylacetamide, DMAP is 4-dimethylaminopyridine, DME is ethyleneglycol dimethyl ether (or 1,2-dimethoxyethane), DMF is N,N-dimethylformamide, DMSO is dimethylsulfoxide, doe is dolaphenine, dov is N,N-dimethylvaline, DTNB is 5,5'-dithiobis(2-nitrobenzoic acid), DTPA is diethylenetriaminepentaacetic acid, DTT is dithiothreitol, EDCI is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EEDQ is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ES-MS is electrospray mass spectrometry, EtOAc is ethyl acetate, Fmoc is N-(9-fluorenylmethoxycarbonyl), gly is glycine, HATU is O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, HOBt is 1-hydroxybenzotriazole, HPLC is high pressure liquid chromatography, ile is isoleucine, lys is lysine, MeCN (CH_3CN) is acetonitrile, MeOH is methanol, Mtr is 4-anisyldiphenylmethyl (or 4-methoxytrityl),nor is (1S, 2R)-(+)-norephedrine, PBS is phosphate-buffered saline (pH 7.4), PEG is polyethylene glycol, Ph is phenyl, Pnp is p-nitrophenyl, MC is 6-maleimidocaproyl, phe is L-phenylalanine, PyBrop is bromo tris-pyrrolidino phosphonium hexafluorophosphate, SEC is size-exclusion chromatography, Su is succinimide, TFA is trifluoroacetic acid, TLC is thin layer chromatography, UV is ultraviolet, and val is valine.

COMPOSITIONS AND METHODS OF MAKING THE SAME

Antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of

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STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as cancer.

Anti-STEAP-1 Antibodies

In one aspect, the invention provides antibodies that bind to STEAP-1. In some embodiments, antibodies are provided that bind to a mature form of human and cynomolgus monkey (cyno) STEAP-1. In one such embodiment, a mature form of human STEAP-1 has an amino acid sequence of SEQ ID NO:1 (Figure 1). The cyno STEAP-1 has an amino acid sequence of SEQ ID NO:3 (Figure 1). In some embodiments, an antibody to STEAP-1 binds to a mature form of STEAP-1 expressed on the cell surface. In some embodiments, an antibody that binds to a mature form of STEAP-1 expressed on the cell surface inhibits the growth of the cell. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and inhibits cell proliferation. In certain embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and induces cell death. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the surface of cancer cells. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 that is overexpressed on the surface of cancer cells relative to normal cells of the same tissue origin. In some embodiments, an anti-STEAP-1 antibody is conjugated to a cytotoxin or a detectable label and binds to STEAP-1 on a cell surface. In some embodiments, the antibody-toxin conjugate inhibits growth of the cell. In some embodiments, the antibody-detectable label conjugate causes a cell expressing STEAP-1 on its surface to be detectable in vitro or in vivo.

In one aspect, an anti-STEAP-1 antibody is a monoclonal antibody. In one aspect, an anti-STEAP-1 antibody is an antibody fragment, e.g., a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an anti-STEAP-1 antibody is a chimeric, humanized, or human antibody. In one aspect, any of the anti-STEAP-1 antibodies described herein are purified.

Exemplary monoclonal antibodies derived from a phage library are provided herein. The antigen used for screening the library was a polypeptide having the sequence of amino acid sequences of SEQ ID NO:28 or SEQ ID NO:30, corresponding to the extracellular domains (ECDs) of STEAP-1 beta and alpha. The antibodies resulting from the library screen are affinity matured.

In one aspect, monoclonal antibodies that compete with murine 120.545, 120 graft, and humanized 120v.24 binding to STEAP-1 are provided. Monoclonal antibodies that bind to the same epitope as murine 120.545, 120 graft, and humanized 120v.24 are also provided.

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In one aspect of the invention, polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the invention, compositions comprising anti-STEAP-1 antibodies or polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, a composition of the invention is a pharmaceutical formulation for the treatment of a cell proliferative disorder, such as those enumerated herein.

A detailed description of exemplary anti-STEAP-1 antibodies is as follows:

1. Specific embodiments of anti-STEAP-1 antibodies

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain comprising SEQ ID NO:9 or 10 of Figure 2B. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising SEQ ID NO:6 of Figure 2A.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain comprising SEQ ID NO:9, having one or more of the following amino changes at the indicated Kabat position: A24V, V37I, V48M, F67I, and L78F. In one embodiment, the heavy chain comprises a heavy chain framework region selected from SEQ ID NOs:25, 75, 76, 77, 78, and 79. As used herein, heavy chain framework regions are designated "FR-H1-H4" or "HC-FR1-FR4," and light chain framework regions are designated "FR-L1-L4" or "LC-FR1-FR4." In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain comprising SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the antibody 120.v24 shown in Figures 2A and 2B.

An anti-STEAP-1 antibody may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind STEAP-1. For example, in some embodiments, anti-STEAP-1 antibodies of the invention comprise a human subgroup III heavy chain framework consensus sequence. In one embodiment of these antibodies, the heavy chain framework consensus sequence comprises substitution(s) at position 24, 37, 48, 67, and/or 78. In one embodiment of these antibodies, position 24 is A or V, position 37 is I or V, position 48 is M or V, position 67 is I or F, and/or position 78 is F or L. In one embodiment, these antibodies comprise a heavy chain variable domain framework sequence of huMAb4D5-8, e.g., SEQ ID NO:21, 22, 23, and 24 (FR-H1, FR-H2, FR-H3, FR-H4, respectively). huMAb4D5-8 is commercially known as HERCEPTIN® anti-HER2 antibody, Genentech, Inc., South San Francisco, CA, USA; also referred to in U.S. Pat. Nos. 6,407,213 & 5,821,337, and Lee et

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al., J. Mol. Biol. (2004), 340(5):1073-93. In one such embodiment, these antibodies further comprise a human κI light chain framework consensus sequence. In one such embodiment, these antibodies comprise a light chain variable domain framework sequence of huMAb4D5-8, e.g. SEQ ID NO:17, 18, 19, and 20 (FR-L1, FR-L2, FR-L3, FR-L4, respectively).

In one embodiment, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the FR-H1-FR-H4 sequences SEQ ID NO:21 or 25 (FR-H1), 22 (FR-H2), 23 (FR-H3), and 24 (FR-H4), respectively; the HVR H1 comprises the amino acid sequence of SEQ ID NO:14; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:15; and the HVR-H3 comprises an amino acid sequence of SEQ ID NO:16. In one embodiment, an anti-STEAP-1 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the FR-L1-FR-L4 sequences of SEQ ID NOs:17, 18, 19 and 20, respectively; the HVR-L1 comprises the amino acid sequence selected from SEQ ID NOs:11, 12, and 13. In one embodiment of these antibodies, the heavy chain variable domain comprises SEQ ID NOs:9 or 10 and the light chain variable domain comprises SEQ ID NO:6.

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:9 or 10. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence SEQ ID NOs:9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, and/or 79. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NO:9 or 10.

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain as depicted in in Figure 2B (SEQ ID NOs:9 or 10).

In some embodiments, the heavy chain HVR and FR sequences comprise the following:

HVR-H1 (GYSITSDYAWN, SEQ ID NO:14)

HVR-H2 (GYISNSGSTSYNPSLKS, SEQ ID NO:15)

HVR-H3 (ERNYDYDDYYYAMDY, SEQ ID NO:16)

FR-H1 (EVQLVESGGGLVQPGGSLRLSCAAS, SEQ ID NO:21)

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FR-H1 (EVQLVESGGGLVQPGGSLRLSCAVS, SEQ ID NO:25)

FR-H2 (WVRQAPGKGLEWV, SEQ ID NO:22)

FR-H2 (WIRQAPGKGLEWV, SEQ ID NO:75)

FR-H2 (WVRQAPGKGLEWM, SEQ ID NO:76)

FR-H2 (WIRQAPGKGLEWM, SEQ ID NO:77)

FR-H3 (RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR, SEQ ID NO:23)

FR-H3 (RITISRDNSKNTLYLQMNSLRAEDTAVYYCAR, SEQ ID NO:78)

FR-H3 (RFTISRDNSKNTFYLQMNSLRAEDTAVYYCAR, SEQ ID NO:79)

FR-H4 (WGQGTLVTVSS, SEQ ID NO:24)

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain as depicted in Figure 2A (SEQ ID NO:6).

In some embodiments, the light chain HVR sequences comprise the following:

HVR-L1(KSSQSLLYRSNQKNYLA, SEQ ID NO:11)

HVR-L2 (WASTRES, SEQ ID NO:12)

HVR-L3 (QQYYNYPRT, SEQ ID NO:13).

In some embodiments, the light chain FR sequences comprise the following:

FR-L1 (DIQMTQSPSSLSASVGDRVTITC, SEQ ID NO:17);

FR-L2 (WYQQKPGKAPKLLIY, SEQ ID NO:18);

FR-L3 (GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC, SEQ ID NO:19)

FR-L4 (FGQGTKVEIKR, SEQ ID NO:20).

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from SEQ ID NOs:6, 11, 12, 13, 17, 18, 19, and 20. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a light chain variable domain comprising the amino acid sequence SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NO:9 and 10;

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and (b) a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in the reference sequence, including but not limited to a sequence selected from SEQ ID NOs:9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, 79. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:9 or 10 and a light chain variable domain comprising an amino acid sequence selected from SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Figure 2B and/or (b) one, two, or three VL HVRs selected from those shown in Figure 2A. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain selected from those shown in Figure 2B and a light chain variable domain selected from those shown in Figure 2A.

2. Antibody Fragments

The present invention encompasses antibody fragments. Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising salvage receptor binding

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epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

3. Humanized Antibodies

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

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

It is further generally desirable that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various

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

4. Human Antibodies

Human anti-STEAP-1 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s) as described above. Alternatively, human monoclonal anti-STEAP-1 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255 (1993); Bruggermann et al., Year in Immunol., 7: 33 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-

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human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

5. Bispecific Antibodies

Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for STEAP-1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of STEAP-1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express STEAP-1. These antibodies possess a STEAP-1-binding arm and an arm which binds a cytotoxic agent, such as, e.g., saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305: 537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker et al., EMBO J., 10: 3655 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion, for example, is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In certain embodiments, the first heavy-chain constant region (CH1), containing the site necessary for light chain binding, is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This

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provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent

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sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

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

6. Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be

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multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. In certain embodiments, the dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. In certain embodiments, a multivalent antibody comprises (or consists of) three to about eight antigen binding sites. In one such embodiment, a multivalent antibody comprises (or consists of) four antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (for example, two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n -VD2-(X2)n -Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein may further comprise at least two (for example, four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

7. Single-Domain Antibodies

In some embodiments, an antibody of the invention is a single-domain antibody. A single-domain antibody is a single polyeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

8. Antibody Variants

In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution

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can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

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

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

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

Addition or deletion of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created or removed. The alteration may also be made by the addition,

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deletion, or substitution of one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and US Patent No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)).

In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US 5739277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (US 2003/0190311, US6821505; US 6165745; US 5624821; US 5648260; US 6165745;US 5834 597).

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

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

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Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (e.g., improved) biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (e.g., the gene III product of M13) packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity). In order to identify candidate hypervariable region sites

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for modification, scanning mutagenesis (e.g., alanine scanning) can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to techniques known in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

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

It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions including that of a hinge cysteine.

In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the invention may comprise one or more alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001). Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants

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with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

In one aspect, the invention provides antibodies comprising modifications in the interface of Fc polypeptides comprising the Fc region, wherein the modifications facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance is positionable in the cavity so as to promote complexing of the first and second Fc polypeptides. Methods of generating antibodies with these modifications are known in the art, e.g., as described in U.S. Pat. No. 5,731,168.

9. Antibody Derivatives

The antibodies of the present invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

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Certain Methods of Making Antibodies

1. Certain Hybridoma-Based Methods

The anti-STEAP-1 monoclonal antibodies of the invention can be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies to STEAP-1 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of STEAP-1 and an adjuvant. STEAP-1 may be prepared using methods well-known in the art, some of which are further described herein. For example, STEAP-1 may be produced recombinantly. In one embodiment, animals are immunized with a derivative of STEAP-1 that contains an extracellular portion of STEAP-1 fused to the Fc portion of an immunoglobulin heavy chain. In one embodiment, animals are immunized with an STEAP-1-IgG1 fusion protein. In one embodiment, animals are immunized with immunogenic derivatives of STEAP-1 in a solution with monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT), and the solution is injected intradermally at multiple sites. Two weeks later the animals are boosted. Seven to fourteen days later the animals are bled, and the serum is assayed for anti- STEAP-1 titer. Animals are boosted until titer plateaus.

Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

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

In certain embodiments, myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell

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Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies that bind to STEAP-1. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

2. *Certain Library Screening Methods*

Anti-STEAP-1 antibodies of the invention can be made by using combinatorial libraries to screen for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are described generally in Hoogenboom et al. (2001) in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ), and in certain embodiments, in Lee et al. (2004) J. Mol. Biol. 340:1073-1093.

In principle, synthetic antibody clones are selected by screening phage libraries containing phage that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are panned by affinity chromatography against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further

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enriched by additional cycles of antigen adsorption/elution. Any of the anti-STEAP-1 antibodies of the invention can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-STEAP-1 antibody clone using the Fv sequences from the phage clone of interest and suitable constant region (Fc) sequences described in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

In certain embodiments, the antigen-binding domain of an antibody is formed from two variable (V) regions of about 110 amino acids, one each from the light (VL) and heavy (VH) chains, that both present three hypervariable loops (HVRs) or complementarity-determining regions (CDRs). Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). As used herein, scFv encoding phage clones and Fab encoding phage clones are collectively referred to as "Fv phage clones" or "Fv clones."

Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

In certain embodiments, filamentous phage is used to display antibody fragments by fusion to the minor coat protein pIII. The antibody fragments can be displayed as single chain Fv fragments, in which VH and VL domains are connected on the same polypeptide chain by a flexible polypeptide spacer, e.g. as described by Marks et al., J. Mol. Biol., 222: 581-597 (1991), or as Fab fragments, in which one chain is fused to pIII and the other is secreted into the bacterial host cell periplasm where assembly of a Fab-coat protein structure which becomes displayed on the phage surface by displacing some of the wild type coat proteins, e.g. as described in Hoogenboom et al., Nucl. Acids Res., 19: 4133-4137 (1991).

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In general, nucleic acids encoding antibody gene fragments are obtained from immune cells harvested from humans or animals. If a library biased in favor of anti-STEAP-1 clones is desired, the subject is immunized with STEAP-1 to generate an antibody response, and spleen cells and/or circulating B cells other peripheral blood lymphocytes (PBLs) are recovered for library construction. In a preferred embodiment, a human antibody gene fragment library biased in favor of anti-STEAP-1 clones is obtained by generating an anti-STEAP-1 antibody response in transgenic mice carrying a functional human immunoglobulin gene array (and lacking a functional endogenous antibody production system) such that STEAP-1 immunization gives rise to B cells producing human antibodies against STEAP-1. The generation of human antibody-producing transgenic mice is described below.

Additional enrichment for anti-STEAP-1 reactive cell populations can be obtained by using a suitable screening procedure to isolate B cells expressing STEAP-1-specific membrane bound antibody, e.g., by cell separation using STEAP-1 affinity chromatography or adsorption of cells to fluorochrome-labeled STEAP-1 followed by flow-activated cell sorting (FACS).

Alternatively, the use of spleen cells and/or B cells or other PBLs from an unimmunized donor provides a better representation of the possible antibody repertoire, and also permits the construction of an antibody library using any animal (human or non-human) species in which STEAP-1 is not antigenic. For libraries incorporating in vitro antibody gene construction, stem cells are harvested from the subject to provide nucleic acids encoding unrearranged antibody gene segments. The immune cells of interest can be obtained from a variety of animal species, such as human, mouse, rat, lagomorpha, luprine, canine, feline, porcine, bovine, equine, and avian species, etc.

Nucleic acid encoding antibody variable gene segments (including VH and VL segments) are recovered from the cells of interest and amplified. In the case of rearranged VH and VL gene libraries, the desired DNA can be obtained by isolating genomic DNA or mRNA from lymphocytes followed by polymerase chain reaction (PCR) with primers matching the 5' and 3' ends of rearranged VH and VL genes as described in Orlandi et al., Proc. Natl. Acad. Sci. (USA), 86: 3833-3837 (1989), thereby making diverse V gene repertoires for expression. The V genes can be amplified from cDNA and genomic DNA, with back primers at the 5' end of the exon encoding the mature V-domain and forward primers based within the J-segment as described in Orlandi et al. (1989) and in Ward et al., Nature, 341: 544-546 (1989). However, for amplifying from cDNA, back primers can also be based in the leader exon as described in Jones et al., Biotechnol., 9: 88-89 (1991), and forward primers within the constant region as described in Sastry et al., Proc. Natl. Acad. Sci. (USA), 86: 5728-5732 (1989). To maximize complementarity, degeneracy can be incorporated in the primers as described in Orlandi et al. (1989) or Sastry et al. (1989). In certain

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embodiments, library diversity is maximized by using PCR primers targeted to each V-gene family in order to amplify all available VH and VL arrangements present in the immune cell nucleic acid sample, e.g. as described in the method of Marks et al., J. Mol. Biol., 222: 581-597 (1991) or as described in the method of Orum et al., Nucleic Acids Res., 21: 4491-4498 (1993). For cloning of the amplified DNA into expression vectors, rare restriction sites can be introduced within the PCR primer as a tag at one end as described in Orlandi et al. (1989), or by further PCR amplification with a tagged primer as described in Clackson et al., Nature, 352: 624-628 (1991).

Repertoires of synthetically rearranged V genes can be derived in vitro from V gene segments. Most of the human VH-gene segments have been cloned and sequenced (reported in Tomlinson et al., J. Mol. Biol., 227: 776-798 (1992)), and mapped (reported in Matsuda et al., Nature Genet., 3: 88-94 (1993); these cloned segments (including all the major conformations of the H1 and H2 loop) can be used to generate diverse VH gene repertoires with PCR primers encoding H3 loops of diverse sequence and length as described in Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). VH repertoires can also be made with all the sequence diversity focused in a long H3 loop of a single length as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 89: 4457-4461 (1992). Human Vκ and Vλ segments have been cloned and sequenced (reported in Williams and Winter, Eur. J. Immunol., 23: 1456-1461 (1993)) and can be used to make synthetic light chain repertoires. Synthetic V gene repertoires, based on a range of VH and VL folds, and L3 and H3 lengths, will encode antibodies of considerable structural diversity. Following amplification of V-gene encoding DNAs, germline V-gene segments can be rearranged in vitro according to the methods of Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

Repertoires of antibody fragments can be constructed by combining VH and VL gene repertoires together in several ways. Each repertoire can be created in different vectors, and the vectors recombined in vitro, e.g., as described in Hogrefe et al., Gene, 128: 119-126 (1993), or in vivo by combinatorial infection, e.g., the loxP system described in Waterhouse et al., Nucl. Acids Res., 21: 2265-2266 (1993). The in vivo recombination approach exploits the two-chain nature of Fab fragments to overcome the limit on library size imposed by E. coli transformation efficiency. Naive VH and VL repertoires are cloned separately, one into a phagemid and the other into a phage vector. The two libraries are then combined by phage infection of phagemid-containing bacteria so that each cell contains a different combination and the library size is limited only by the number of cells present (about 10^{12} clones). Both vectors contain in vivo recombination signals so that the VH and VL genes are recombined onto a single replicon and are co-packaged into phage virions. These huge libraries provide large numbers of diverse antibodies of good affinity (K_d^{-1} of about 10^{-8} M).

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Alternatively, the repertoires may be cloned sequentially into the same vector, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 88: 7978-7982 (1991), or assembled together by PCR and then cloned, e.g. as described in Clackson et al., Nature, 352: 624-628 (1991). PCR assembly can also be used to join VH and VL DNAs with DNA encoding a flexible peptide spacer to form single chain Fv (scFv) repertoires. In yet another technique, "in cell PCR assembly" is used to combine VH and VL genes within lymphocytes by PCR and then clone repertoires of linked genes as described in Embleton et al., Nucl. Acids Res., 20: 3831-3837 (1992).

The antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_d^{-1} of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked in vitro by constructing and reselecting from secondary libraries as described in Winter et al. (1994), supra. For example, mutation can be introduced at random in vitro by using error-prone polymerase (reported in Leung et al., Technique, 1: 11-15 (1989)) in the method of Hawkins et al., J. Mol. Biol., 226: 889-896 (1992) or in the method of Gram et al., Proc. Natl. Acad. Sci USA, 89: 3576-3580 (1992). Additionally, affinity maturation can be performed by randomly mutating one or more CDRs, e.g. using PCR with primers carrying random sequence spanning the CDR of interest, in selected individual Fv clones and screening for higher affinity clones. WO 9607754 (published 14 March 1996) described a method for inducing mutagenesis in a complementarity determining region of an immunoglobulin light chain to create a library of light chain genes. Another effective approach is to recombine the VH or VL domains selected by phage display with repertoires of naturally occurring V domain variants obtained from unimmunized donors and screen for higher affinity in several rounds of chain reshuffling as described in Marks et al., Biotechnol., 10: 779-783 (1992). This technique allows the production of antibodies and antibody fragments with affinities of about 10^{-9} M or less.

Screening of the libraries can be accomplished by various techniques known in the art. For example, STEAP-1 can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning phage display libraries.

The phage library samples are contacted with immobilized STEAP-1 under conditions suitable for binding at least a portion of the phage particles with the adsorbent. Normally, the conditions, including pH, ionic strength, temperature and the like are selected to mimic physiological conditions. The phages bound to the solid phase are washed and then eluted by acid, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci USA, 88: 7978-7982 (1991), or by alkali, e.g. as described in Marks et al., J. Mol. Biol., 222: 581-597 (1991), or by STEAP-1 antigen competition, e.g. in a procedure similar to the antigen competition

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method of Clackson et al., Nature, 352: 624-628 (1991). Phages can be enriched 20-1,000-fold in a single round of selection. Moreover, the enriched phages can be grown in bacterial culture and subjected to further rounds of selection.

The efficiency of selection depends on many factors, including the kinetics of dissociation during washing, and whether multiple antibody fragments on a single phage can simultaneously engage with antigen. Antibodies with fast dissociation kinetics (and weak binding affinities) can be retained by use of short washes, multivalent phage display and high coating density of antigen in solid phase. The high density not only stabilizes the phage through multivalent interactions, but favors rebinding of phage that has dissociated. The selection of antibodies with slow dissociation kinetics (and good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass et al., Proteins, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks et al., Biotechnol., 10: 779-783 (1992).

It is possible to select between phage antibodies of different affinities, even with affinities that differ slightly, for STEAP-1. However, random mutation of a selected antibody (e.g. as performed in some affinity maturation techniques) is likely to give rise to many mutants, most binding to antigen, and a few with higher affinity. With limiting STEAP-1, rare high affinity phage could be competed out. To retain all higher affinity mutants, phages can be incubated with excess biotinylated STEAP-1, but with the biotinylated STEAP-1 at a concentration of lower molarity than the target molar affinity constant for STEAP-1. The high affinity-binding phages can then be captured by streptavidin-coated paramagnetic beads. Such "equilibrium capture" allows the antibodies to be selected according to their affinities of binding, with sensitivity that permits isolation of mutant clones with as little as two-fold higher affinity from a great excess of phages with lower affinity. Conditions used in washing phages bound to a solid phase can also be manipulated to discriminate on the basis of dissociation kinetics.

Anti-STEAP-1 clones may be selected based on activity. In certain embodiments, the invention provides anti-STEAP-1 antibodies that bind to living cells that naturally express STEAP-1. In one embodiment, the invention provides anti-STEAP-1 antibodies that block the binding between a STEAP-1 ligand and STEAP-1, but do not block the binding between a STEAP-1 ligand and a second protein. Fv clones corresponding to such anti-STEAP-1 antibodies can be selected by (1) isolating anti-STEAP-1 clones from a phage library as described above, and optionally amplifying the isolated population of phage clones by growing up the population in a suitable bacterial host; (2) selecting STEAP-1 and a second protein against which blocking and non-blocking activity, respectively, is desired; (3) adsorbing the anti-STEAP-1 phage clones to immobilized STEAP-1; (4) using an excess of the second protein to elute any

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undesired clones that recognize STEAP-1-binding determinants which overlap or are shared with the binding determinants of the second protein; and (5) eluting the clones which remain adsorbed following step (4). Optionally, clones with the desired blocking/non-blocking properties can be further enriched by repeating the selection procedures described herein one or more times.

DNA encoding hybridoma-derived monoclonal antibodies or phage display Fv clones of the invention is readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide primers designed to specifically amplify the heavy and light chain coding regions of interest from hybridoma or phage DNA template). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of the desired monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs, 130: 151 (1992).

DNA encoding the Fv clones of the invention can be combined with known DNA sequences encoding heavy chain and/or light chain constant regions (e.g. the appropriate DNA sequences can be obtained from Kabat et al., supra) to form clones encoding full or partial length heavy and/or light chains. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. An Fv clone derived from the variable domain DNA of one animal (such as human) species and then fused to constant region DNA of another animal species to form coding sequence(s) for "hybrid," full length heavy chain and/or light chain is included in the definition of "chimeric" and "hybrid" antibody as used herein. In certain embodiments, an Fv clone derived from human variable DNA is fused to human constant region DNA to form coding sequence(s) for full- or partial-length human heavy and/or light chains.

DNA encoding anti-STEAP-1 antibody derived from a hybridoma of the invention can also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of homologous murine sequences derived from the hybridoma clone (e.g. as in the method of Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). DNA encoding a hybridoma- or Fv clone-derived antibody or fragment can be further modified by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In this manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the Fv clone or hybridoma clone-derived antibodies of the invention.

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3. Vectors, Host Cells, and Recombinant Methods

For recombinant production of an antibody of the invention, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating antibodies using prokaryotic host cells:

Vector Construction

Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically transformed using pBR322, a plasmid derived from an E. coli species. pBR322 contains genes encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial

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plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Patent No. 5,648,237.

In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λGEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as E. coli LE392.

The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β-galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For

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prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

In another aspect, the production of the immunoglobulins according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB- strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

Antibodies of the invention can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a "codon bank" at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) METHODS: A Companion to Methods in Enzymol. 4:151-158.

In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of

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the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5, 840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the invention.

Prokaryotic host cells suitable for expressing antibodies of the invention include Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., E. coli), Bacilli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the invention. Examples of E. coli strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli B, E. coliλ 1776 (ATCC 31,537) and E. coli RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

Antibody Production

Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

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Prokaryotic cells used to produce the polypeptides of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for E. coli growth, growth temperatures range from about 20°C to about 39°C; from about 25°C to about 37°C; or about 30°C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for E. coli, the pH is from about 6.8 to about 7.4, or about 7.0.

If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al., J. Immunol. Methods (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

In one embodiment, the expressed polypeptides of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

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In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Patent No. 6,083,715; Georgiou et al., U.S. Patent No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Patent No. 5,264,365; Georgiou et al., U.S. Patent No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

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In one embodiment, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

Antibody Purification

In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the invention. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating antibodies using eukaryotic host cells:

A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

Signal sequence component

A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as

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viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

Origin of replication

Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

Selection gene component

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

Promoter component

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Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (e.g., an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982), describing expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

Enhancer element component

Transcription of DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is generally located at a site 5' from the promoter.

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Transcription termination component

Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

Selection and transformation of host cells

Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)) ; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Culturing the host cells

The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for

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the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Purification of antibody

When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

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Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Immunoconjugates

The invention also provides immunoconjugates (interchangeably referred to as "antibody-drug conjugates," or "ADCs") comprising any of the anti-STEAP-1 antibodies of the invention conjugated to one or more cytotoxic agents, such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and a chemotherapeutic agent or other toxin. Chemotherapeutic agents useful in the generation of immunoconjugates are described herein (e.g., above). Enzymatically active toxins and fragments thereof can also be used and are described herein.

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and one or more small molecule toxins, including, but not limited to, small molecule drugs such as a calicheamicin, maytansinoid, dolastatin, auristatin, trichothecene, and CC1065, and the derivatives of these drugs that have cytotoxic activity. Examples of such immunoconjugates are discussed in further detail below.

1. Exemplary Immunoconjugates

An immunoconjugate (or "antibody-drug conjugate" ("ADC")) of the invention may be of Formula I, below, wherein an anti-STEAP-1 antibody is conjugated (i.e., covalently attached) to one or more drug moieties (D) through an optional linker (L).

$$Ab–(L–D)_p \qquad \text{Formula I}$$

Accordingly, the anti-STEAP-1 antibody may be conjugated to the drug either directly or via a linker. In Formula I, p is the average number of drug moieties per antibody, which can range, e.g., from

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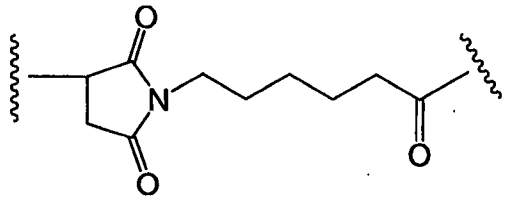
about 1 to about 20 drug moieties per antibody, and in certain embodiments, from 1 to about 8 drug moieties per antibody.

Exemplary Linkers

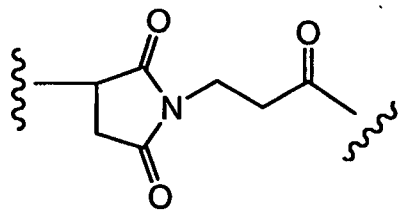
Exemplary linkers and drug moieties are disclosed herein. A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). Various linker components are known in the art, some of which are described below.

A linker may be a "cleavable linker," facilitating release of a drug in the cell. For example, an acid-labile linker (e.g., hydrazone), protease-sensitive (e.g., peptidase-sensitive) linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

In some embodiments, a linker component may comprise a "stretcher unit" that links an antibody to another linker component or to a drug moiety. Exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody):

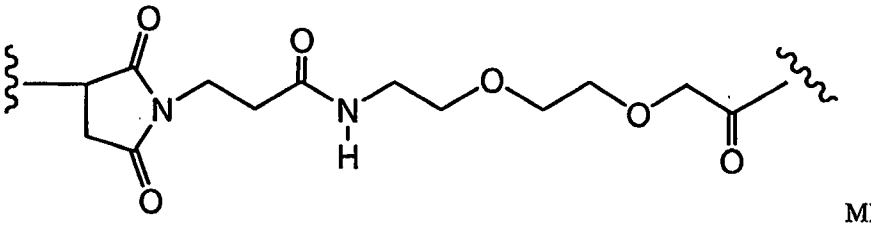


MC

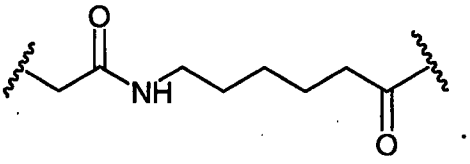


MP

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MPEG



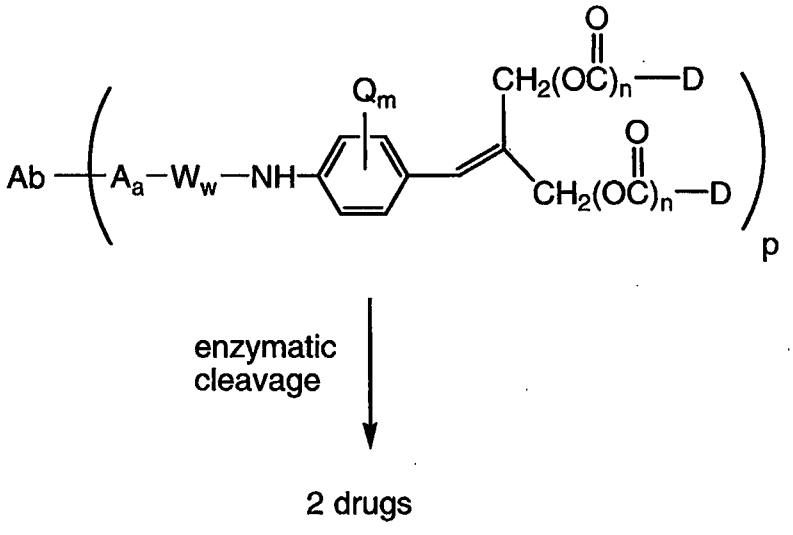
In some embodiments, a linker component may comprise an amino acid unit. In one such embodiment, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes. See, e.g., Doronina et al. (2003) Nat. Biotechnol. 21:778-784. Exemplary amino acid units include, but are not limited to, a dipeptide, a tripeptide, a tetrapeptide, and a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); or N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

In some embodiments, a linker component may comprise a "spacer" unit that links the antibody to a drug moiety, either directly or by way of a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon enzymatic (e.g., proteolytic) cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. Other combinations of peptidic spacers susceptible to sequence-specific enzymatic cleavage are also contemplated. For example, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease would result in release of a glycine-glycine-drug moiety from the remainder of the ADC. In one such embodiment, the glycine-glycine-drug moiety is then subjected to a separate hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

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A "self-immolative" spacer unit allows for release of the drug moiety without a separate hydrolysis step. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In one such embodiment, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and a cytotoxic agent. See, e.g., Hamann et al. (2005) Expert Opin. Ther. Patents (2005) 15:1087-1103. In one embodiment, the spacer unit is p-aminobenzyloxycarbonyl (PAB). In certain embodiments, the phenylene portion of a p-amino benzyl unit is substituted with Qm, wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen,- nitro or -cyano; and m is an integer ranging from 0-4. Examples of self-immolative spacer units further include, but are not limited to, aromatic compounds that are electronically similar to p-aminobenzyl alcohol (see, e.g., US 2005/0256030 A1), such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho- or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., Chemistry Biology, 1995, 2, 223); appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, et al., J. Amer. Chem. Soc., 1972, 94, 5815); and 2-aminophenylpropionic acid amides (Amsberry, et al., J. Org. Chem., 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the a-position of glycine (Kingsbury, et al., J. Med. Chem., 1984, 27, 1447) are also examples of self-immolative spacers useful in ADCs.

In one embodiment, a spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted below, which can be used to incorporate and release multiple drugs.



wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

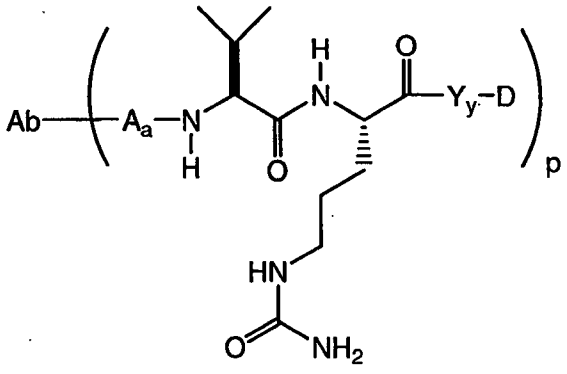
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A linker may comprise any one or more of the above linker components. In certain embodiments, a linker is as shown in brackets in the following ADC Formula II

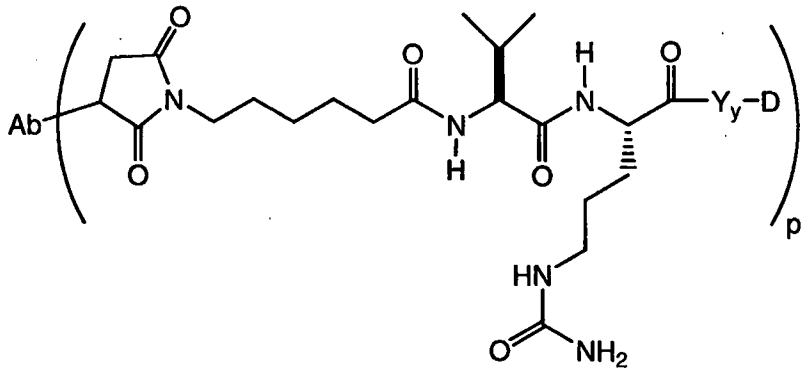
$Ab-([Aa-Ww-Yy]-D)_p$ Formula II

wherein A is a stretcher unit, and a is an integer from 0 to 1; W is an amino acid unit, and w is an integer from 0 to 12; Y is a spacer unit, and y is 0, 1, or 2; and Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in US 20050238649 A1, which is expressly incorporated herein by reference.

Exemplary linker components and combinations thereof are shown below in the context of ADCs of Formula II:

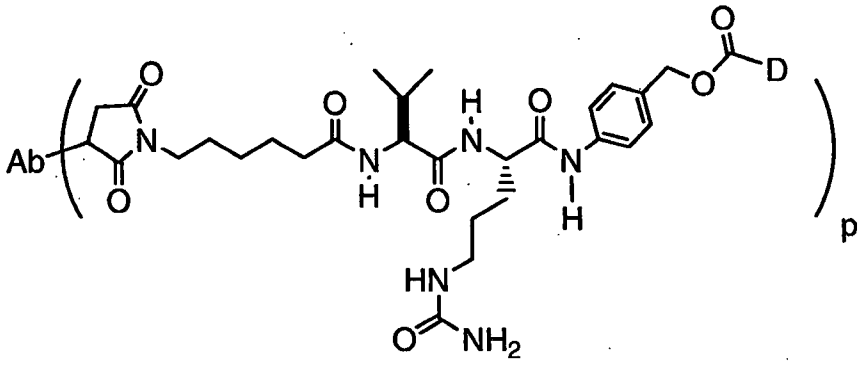


Val-Cit or VC



MC-val-cit

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MC-val-cit-PAB

Linkers components, including stretcher, spacer, and amino acid units, may be synthesized by methods known in the art, such as those described in US 2005-0238649 A1.

Exemplary Drug Moieties

Maytansine and maytansinoids

In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to one or more maytansinoid molecules. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

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 or produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973). Maytansinol and maytansinol analogues may also be prepared synthetically according to known methods.

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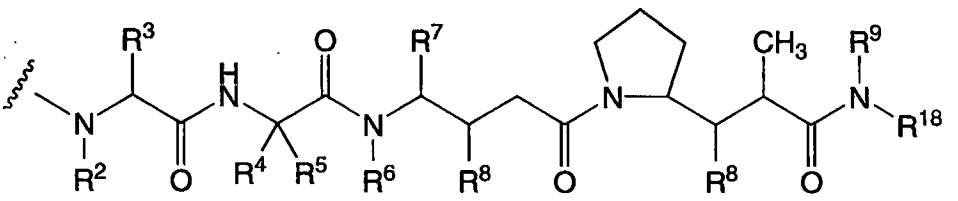
Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, as disclosed herein.

Auristatins and dolastatins

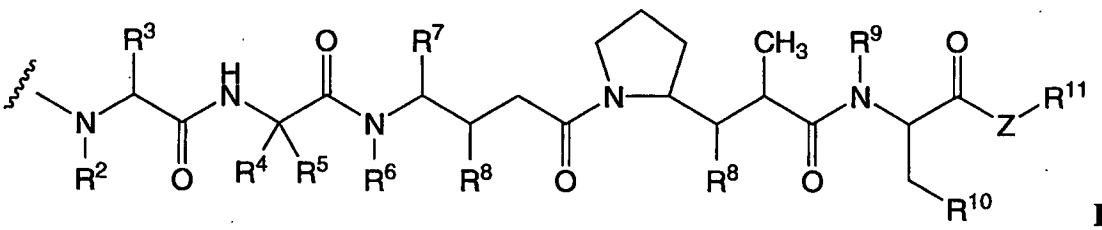
In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to dolastatin or a dolastatin peptidic analog or derivative, e.g., an auristatin (US Pat. Nos. 5635483; 5780588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (US Pat. No.5663149) 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).

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 March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

A peptidic drug moiety may be selected from Formulas D_E and D_F below:



D_E



D_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

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R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O-(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$-C_8 alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2-$aryl, $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment, R^3, R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is –H or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is -H, and R^7 is sec-butyl.

In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is -H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is -H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is -H.

In one embodiment, Z is -O- or -NH-.

In one embodiment, R^{10} is aryl.

In an exemplary embodiment, R^{10} is -phenyl.

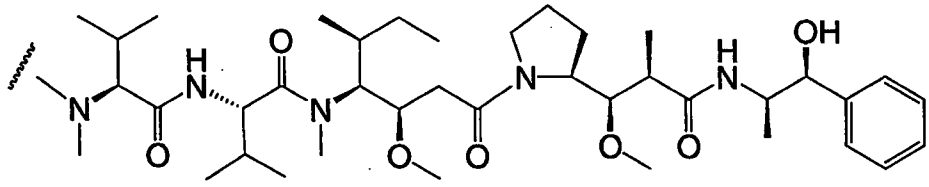
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In an exemplary embodiment, when Z is -O-, R^{11} is –H, methyl or t-butyl.

In one embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

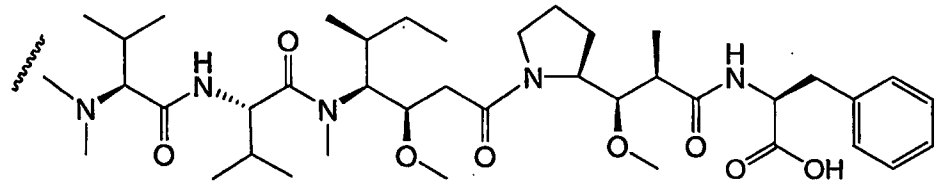
In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



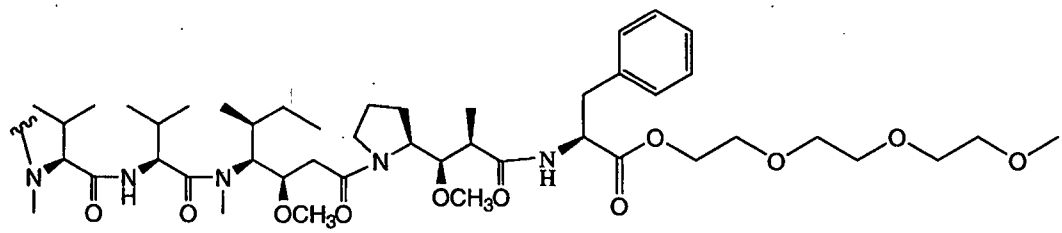
MMAE

An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate (*see* US 2005/0238649 and Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124):

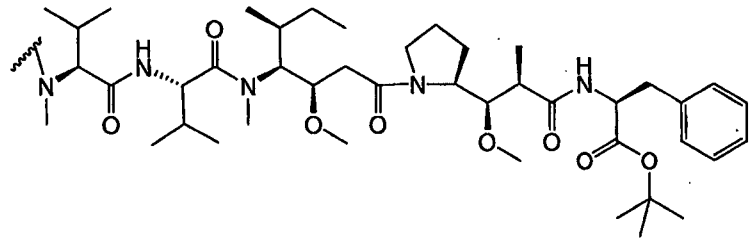


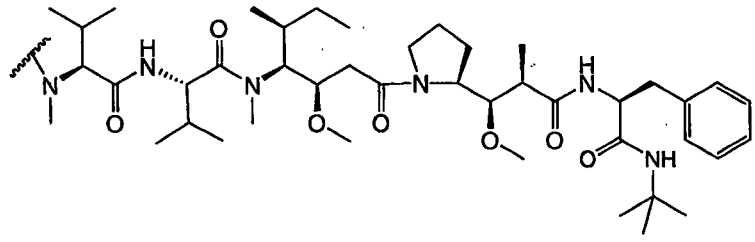
MMAF

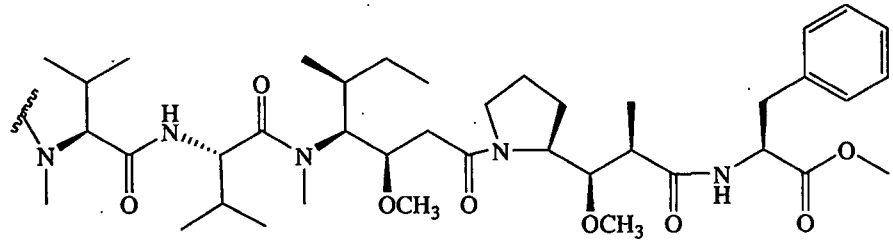
Other drug moieties include the following MMAF derivatives, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

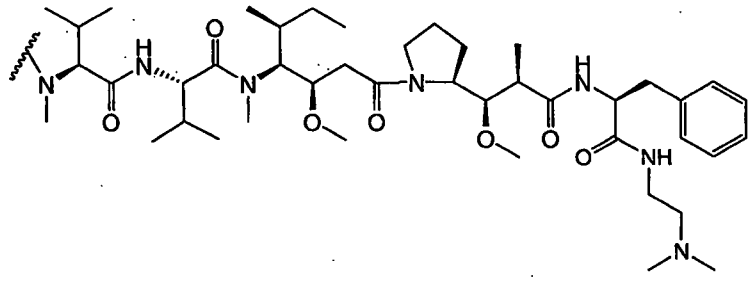


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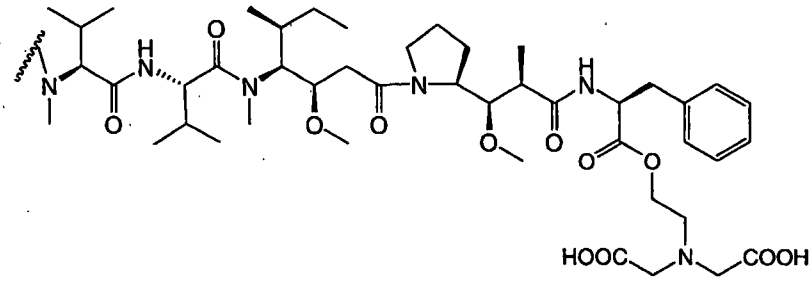




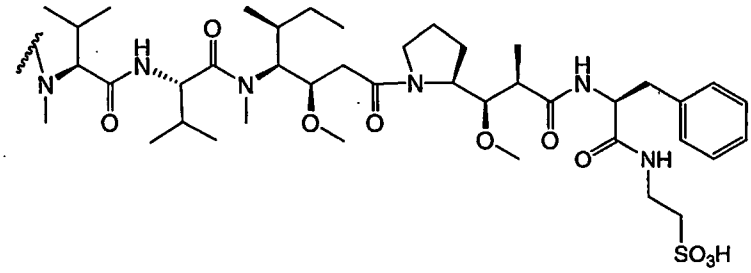




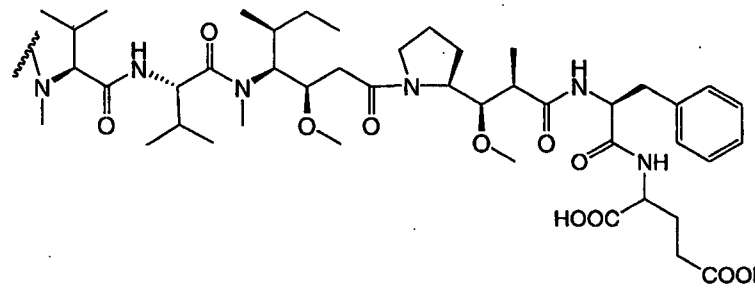
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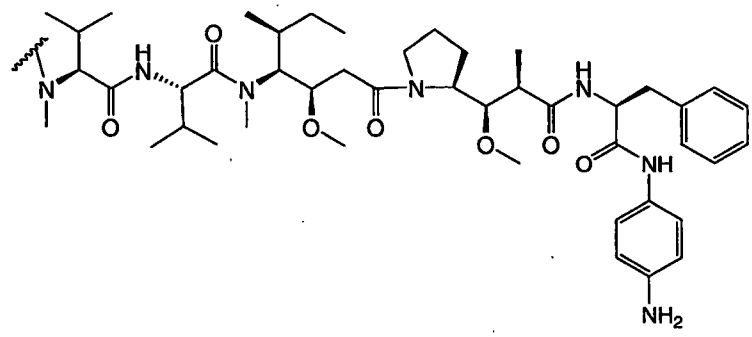
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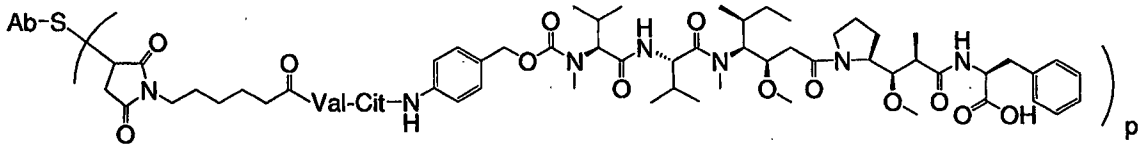
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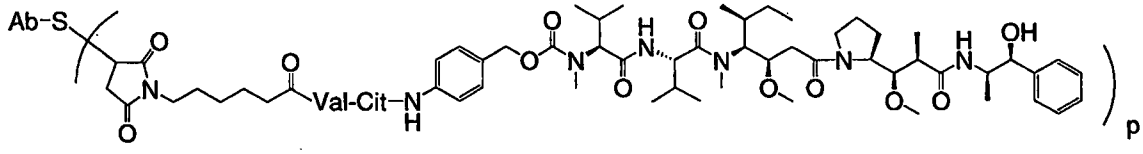
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In one aspect, hydrophilic groups including but not limited to, triethylene glycol esters (TEG), as shown above, can be attached to the drug moiety at R^{11}. Without being bound by any particular theory, the hydrophilic groups assist in the internalization and non-agglomeration of the drug moiety.

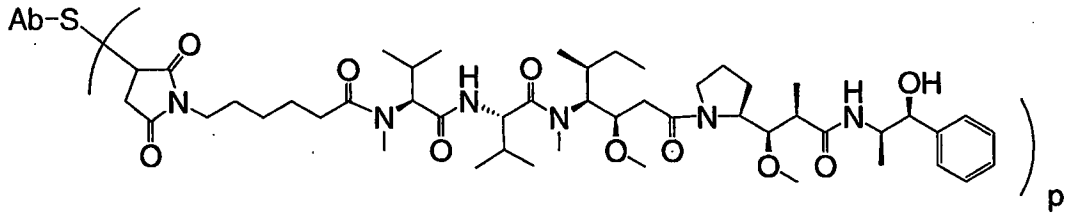
Exemplary embodiments of ADCs of Formula I comprising an auristatin/dolastatin or derivative thereof are described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124, which is expressly incorporated herein by reference. Exemplary embodiments of ADCs of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein "Ab" is an antibody; p is 1 to about 8, "Val-Cit" is a valine-citrulline dipeptide; and "S" is a sulfur atom:



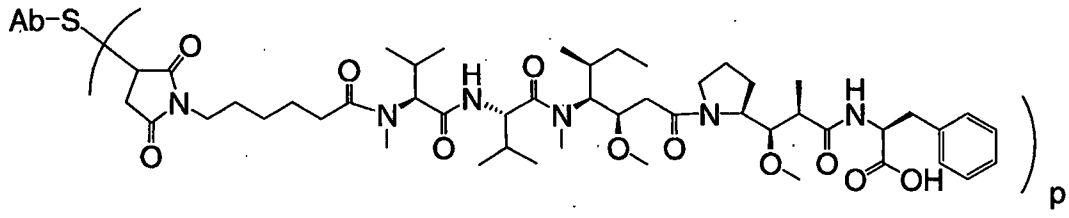
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

Exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Interestingly, immunoconjugates comprising

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MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker. See, Doronina et al. (2006) Bioconjugate Chem. 17:114-124. In such instances, drug release is believed to be effected by antibody degradation in the cell. Id.

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. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. Auristatin/dolastatin drug moieties may be prepared according to the methods of: US 2005-0238649 A1; US Pat. No.5635483; US Pat. No.5780588; 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.

In particular, auristatin/dolastatin drug moieties of formula D_F, such as MMAF and derivatives thereof, may be prepared using methods described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124. Auristatin/dolastatin drug moieties of formula D_E, such as MMAE and derivatives thereof, may be prepared using methods described in Doronina et al. (2003) Nat. Biotech. 21:778-784. Drug-linker moieties MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE may be conveniently synthesized by routine methods, e.g., as described in Doronina et al. (2003) Nat. Biotech. 21:778-784, and Patent Application Publication No. US 2005/0238649 A1, and then conjugated to an antibody of interest.

Drug Loading

Drug loading is represented by p and is the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I 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, ELISA assay, and HPLC. 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 reverse phase HPLC or electrophoresis.

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,

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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).

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.

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)).

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 (see, e.g., Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of

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an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Certain Methods of Preparing Immunconjugates

An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in US 20050238649 A1, which is expressly incorporated herein by reference.

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and

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arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic subsituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

Nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A; see pages 467-498, 2003-2004 Applications Handbook and Catalog.

Immunoconjugates comprising an antibody and a cytotoxic agent may also be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene

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triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

Alternatively, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

Pharmaceutical Formulations

Administration of Antibody-Drug Conjugates

The antibody-drug conjugates (ADC) of the invention may be administered by any route appropriate to the condition to be treated. The ADC will typically be administered parenterally, i.e. infusion, subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural.

For treating cancers of, for example, prostate, lung and/or colon, in one embodiment, the antibody-drug conjugate is administered via intravenous infusion. The dosage administered via infusion is in the range of about 1 $\mu g/m^2$ to about 10,000 $\mu g/m^2$ per dose, generally one dose per week for a total of one, two, three or four doses. Alternatively, the dosage range is of about 1 $\mu g/m^2$ to about 1000 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 800 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 600 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 400 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 500 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 300 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 200 $\mu g/m^2$, and about 1 $\mu g/m^2$ to about 200 $\mu g/m^2$. The dose may be administered once per day, once per week, multiple times per week, but less than once per day, multiple times per month but less than once per day, multiple times per month but less than once per week, once per month or intermittently to relieve or alleviate symptoms of the disease. Administration may continue at any of the disclosed intervals until remission of the tumor or symptoms of the lymphoma, leukemia being treated. Administration may continue after remission or relief of symptoms is achieved where such remission or relief is prolonged by such continued administration.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding

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embodiments, which antibody is not conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding embodiments, which antibody is conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

In one aspect, the invention further provides pharmaceutical formulations comprising at least one anti-STEAP-1 antibody of the invention and/or at least one immunoconjugate thereof and/or at least one anti-STEAP-1 antibody-drug conjugate of the invention. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an anti-STEAP-1 antibody-drug conjugate and/or an immunoconjugate thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an immunoconjugate thereof, and optionally, 2) at least one additional therapeutic agent.

Pharmaceutical formulations comprising an antibody or immunoconjugate of the invention or the antibody-drug conjugate of the invention are prepared for storage by mixing the antibody or antibody-drug conjugate having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)) in the form of aqueous solutions or lyophilized or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, histidine 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). Pharmaceutical formulations to be used for in vivo administration are generally sterile. This is readily accomplished by filtration through sterile filtration membranes.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate of the invention, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. 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. When encapsulated antibodies or immunoconjugates remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, 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.

Antibody-Drug Conjugate Treatments

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders. Still others include prostate, lung and colon cancers.

The ADC compounds which are identified in the animal models and cell-based assays can be further tested in tumor-bearing higher primates and human clinical trials. Human clinical trials can be

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designed to test the efficacy of the anti-STEAP-1 monoclonal antibody or immunoconjugate of the invention in patients experiencing a prostate, lung or colon cell proliferative disorder including without limitation prostate, lung and colon cancers and metastases of such cancers. The clinical trial may be designed to evaluate the efficacy of an ADC in combinations with known therapeutic regimens, such as radiation and/or chemotherapy involving known chemotherapeutic and/or cytotoxic agents.

The cancer may comprise STEAP-1-expressing cells, such that an ADC of the present invention is able to bind to the cancer cells. To determine STEAP-1 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, STEAP-1 overexpression may be analyzed by IHC. Parrafin-embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a STEAP-1 protein staining intensity criteria with respect to the degree of staining and in what proportion of tumor cells examined.

For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight.

For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of an anti-STEAP-1 antibody. Other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Combination Therapy

An antibody-drug conjugate (ADC) of the invention may be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with at least one additional compound having anti-cancer properties. The at lelast one additional compound of the pharmaceutical

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combination formulation or dosing regimen preferably has complementary activities to the ADC of the combination such that they do not adversely affect each other.

The at least one additional compound may be a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. A pharmaceutical composition containing an ADC of the invention may also have a therapeutically effective amount of a chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, or a DNA binder.

In one aspect, the first compound is an anti-STEAP-1 ADC of the invention and the at least one additional compound is a therapeutic antibody other than an anti-STEAP-1 (naked antibody or an ADC). In one embodiment, the at least one additional compound is an anti-PSCA antibody. In one embodiment the at least one additional compound is an anti-HER2 antibody, trastuzumab (e.g., Herceptin®, Genentech, Inc., South San Francisco, CA). In one embodiment the at least one additional compound is an anti-HER2 antibody, pertuzumab (Omnitarg™, Genentech, Inc., South San Francisco, CA, see US6949245). In one embodiment, the at least one additional compound is an anti-VEGF antibody (e.g, Avastin®, Genentech, Inc.). In each case, the at least one compound is either a naked antibody or an ADC). In an embodiment, the at least one additional compound is an antibody (either a naked antibody or an ADC), and the additional antibody is a second, third, fourth, fifth, sixth antibody or more, such that a combination of such second, third, fourth, fifth, sixth, or more antibodies (either naked or as an ADC) is efficacious in treating a cell proliferative disease in a tissue expressing STEAP-1.

Other therapeutic regimens may be combined with the administration of an anticancer agent identified in accordance with this invention, including without limitation radiation therapy and/or bone marrow and peripheral blood transplants, and/or a cytotoxic agent, a chemotherapeutic agent, or a growth inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA, anti-HER2 (e.g., Herceptin®, Omnitarg™) or anti-VEGF (e.g., Avastin®). The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

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In one embodiment, treatment with an ADC involves the combined administration of an anticancer agent identified herein, and one or more chemotherapeutic agents or growth inhibitory agents, including coadministration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include taxanes (such as paclitaxel and docetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in "Chemotherapy Service", (1992) Ed., M.C. Perry, Williams & Wilkins, Baltimore, Md.

Suitable dosages for any of the above coadministered agents are those presently used and may be lowered due to the combined action (synergy) of the newly identified agent and other chemotherapeutic agents or treatments.

The combination therapy may provide "synergy" and prove "synergistic", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Metabolites of the Antibody-Drug Conjugates

Also falling within the scope of this invention are the in vivo metabolic products of the ADC compounds described herein, to the extent such products are novel and unobvious over the prior art. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification, enzymatic cleavage, and the like, of the administered compound. Accordingly, the invention includes novel and unobvious compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof.

Metabolite products typically are identified by preparing a radiolabelled (e.g. 14C or 3H) ADC, administering it parenterally in a detectable dose (e.g. greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other

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biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g. by MS, LC/MS or NMR analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found in vivo, are useful in diagnostic assays for therapeutic dosing of the ADC compounds of the invention.

Further Methods of Using Anti-STEAP-1 Antibodies and Immunoconjugates

Diagnostic methods and methods of detection

In one aspect, anti-STEAP-1 antibodies and immunoconjugates of the invention are useful for detecting the presence of STEAP-1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue. In certain embodiments, such tissues include normal and/or cancerous tissues that express STEAP-1 at higher levels relative to other tissues, for example, prostate, lung and colon.

In one aspect, the invention provides a method of detecting the presence of STEAP-1 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1.

In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of STEAP-1. In certain embodiments, the method comprises contacting a test cell with an anti-STEAP-1 antibody; determining the level of expression (either quantitatively or qualitatively) of STEAP-1 by the test cell by detecting binding of the anti-STEAP-1 antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell (e.g., a normal cell of the same tissue origin as the test cell or a cell that expresses STEAP-1 at levels comparable to such a normal cell), wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of STEAP-1. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of STEAP-1. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor.

Exemplary cell proliferative disorders that may be diagnosed using an antibody of the invention include a prostate, lung and colon cancers or metastases of such cancers.

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In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface. In certain embodiments, the method comprises contacting a cell with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1 on the cell surface. An exemplary assay for detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed STEAP-1 on the surface of a cell is a "FACS" assay.

Certain other methods can be used to detect binding of anti-STEAP-1 antibodies to STEAP-1. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

In certain embodiments, anti-STEAP-1 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

In certain embodiments, anti-STEAP-1 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-STEAP-1 antibody from any STEAP-1 that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-STEAP-1 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al.., U.S. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-STEAP-1 antibody after formation of a complex between the anti-STEAP-1 antibody and STEAP-1, e.g., by immunoprecipitation.

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Any of the above embodiments of diagnosis or detection may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-STEAP-1 antibody.

Therapeutic methods

An antibody or immunoconjugate of the invention may be used in, for example, in vitro, ex vivo, and in vivo therapeutic methods. In one aspect, the invention provides methods for inhibiting cell growth or proliferation, either in vivo or in vitro, the method comprising exposing a cell to an anti-STEAP-1 antibody or immunoconjugate thereof under conditions permissive for binding of the immunoconjugate to STEAP-1. "Inhibiting cell growth or proliferation" means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death. In certain embodiments, the cell is a tumor cell. In certain embodiments, the cell is a prostate, lung, colon, bladder, or ovary cell, or Ewing's sarcoma cell. In certain embodiments, the cell is a xenograft, e.g., as exemplified herein.

In one aspect, an antibody or immunoconjugate of the invention is used to treat or prevent a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder. In certain embodiments, the cell proliferative disorder is associated with increased expression and/or activity of STEAP-1. For example, in certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell. In certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is a tumor or a cancer or metastasis of such cancer.

In one aspect, the invention provides methods for treating a prostate, lung or colon cell proliferative disorder comprising administering to an individual an effective amount of an anti-STEAP-1 antibody or immunoconjugate thereof. In certain embodiments, a method for treating a prostate, lung or colon cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising an anti-STEAP-1 antibody or anti-STEAP-1 immunoconjugate and, optionally, at least one additional therapeutic agent, such as those provided herein.

In one aspect, at least some of the antibodies or immunoconjugates of the invention can bind STEAP-1 from species other than human. Accordingly, antibodies or immunoconjugates of the invention can be used to bind STEAP-1, e.g., in a cell culture containing STEAP-1, in humans, or in other mammals having a STEAP-1 with which an antibody or immunoconjugate of the invention cross-reacts (e.g. chimpanzee, baboon, marmoset, cynomolgus and rhesus monkeys, dog, pig, rat, or mouse). In one

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embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used for targeting STEAP-1 on prostate, lung or colono cells by contacting the antibody or immunoconjugate with STEAP-1 to form an antibody or immunoconjugate-antigen complex such that a conjugated cytotoxin of the immunoconjugate accesses the interior of the cell. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is human STEAP-1. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is cynomolgus monkey STEAP-1. In one embodiment, the humanized anti-STEAP-1 antibody binds to human and/or cynomolgus monkey STEAP-1.

In one embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used in a method for binding STEAP-1 in an individual suffering from a disorder associated with increased STEAP-1 expression and/or activity, the method comprising administering to the individual the antibody or immunoconjugate such that STEAP-1 in the individual is bound. In one embodiment, the bound antibody or immunoconjugate is internalized into the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell expressing STEAP-1. In one embodiment, the STEAP-1 is human STEAP-1, and the individual is a human individual. Alternatively, the individual can be a mammal expressing STEAP-1 to which an anti-STEAP-1 antibody binds. Still further the individual can be a mammal into which STEAP-1 has been introduced (e.g., by administration of STEAP-1 or by expression of a transgene encoding STEAP-1).

An anti-STEAP-1 antibody or immunoconjugate can be administered to a human for therapeutic purposes. Moreover, an anti-STEAP-1 antibody or immunoconjugate can be administered to a non-human mammal expressing STEAP-1 with which the antibody cross-reacts (e.g., a primate, dog, pig, rat, or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies or immunoconjugates of the invention (e.g., testing of dosages and time courses of administration).

Antibodies or immunoconjugates of the invention can be used either alone or in combination with other compositions in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent and/or adjuvant. In certain embodiments, an additional therapeutic agent is a cytotoxic agent, a chemotherapeutic agent, or a growth inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA (see, for example, US6824780), anti-VEGF (e.g., Avastin®, Genentech, Inc.), anti-HER2 (e.g., Herceptin®, Omnitarg™ Genentech, Inc.), or anti-HER2 in combination with Taxol® (see, for example, BioWorld Today,

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November 17, 1999, page 1), wherein the combination therapy is useful in the treatment of cell proliferative disorders, cancers, and/or metastases of cancers of prostate, lung and/or colon.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

An antibody or immunoconjugate of the invention (and any additional therapeutic agent or adjuvant) can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody or immunoconjugate is suitably administered by pulse infusion, particularly with declining doses of the antibody or immunoconjugate. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or immunoconjugate present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents, such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of antibody or immunoconjugate, the severity and course of the disease, whether the antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the discretion of the attending

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physician. The antibody or immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg (e.g. 0.1mg/kg-20mg/kg) of antibody or immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) of antibody or immunoconjugate may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Assays

Anti-STEAP-1 antibodies and immunoconjugates of the invention may be characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Activity assays

In one aspect, assays are provided for identifying anti-STEAP-1 antibodies or immunoconjugates thereof having biological activity. Biological activity may include, e.g., the ability to inhibit cell growth or proliferation (e.g., "cell killing" activity), or the ability to induce cell death, including programmed cell death (apoptosis). Antibodies or immunoconjugates having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vitro. Assays for inhibition of cell growth or proliferation are well known in the art. Certain assays for cell proliferation, exemplified by the "cell killing" assays described herein, measure cell viability. One such assay is the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically

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active cells. See Crouch et al (1993) J. Immunol. Meth. 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). See Cree et al (1995) AntiCancer Drugs 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

Another assay for cell proliferation is the "MTT" assay, a colorimetric assay that measures the oxidation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by mitochondrial reductase. Like the CellTiter-Glo™ assay, this assay indicates the number of metabolically active cells present in a cell culture. See, e.g., Mosmann (1983) J. Immunol. Meth. 65:55-63, and Zhang et al. (2005) Cancer Res. 65:3877-3882.

In one aspect, an anti-STEAP-1 antibody is tested for its ability to induce cell death in vitro. Assays for induction of cell death are well known in the art. In some embodiments, such assays measure, e.g., loss of membrane integrity as indicated by uptake of propidium iodide (PI), trypan blue (see Moore et al. (1995) Cytotechnology, 17:1-11), or 7AAD. In an exemplary PI uptake assay, cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. Cells are seeded at a density of 3 x 10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is removed and replaced with fresh medium alone or medium containing various concentrations of the antibody or immunoconjugate. The cells are incubated for a 3-day time period. Following treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Antibodies or immunoconjugates which induce statistically significant levels of cell death as determined by PI uptake are thus identified.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate is tested for its ability to induce apoptosis (programmed cell death) in vitro. An exemplary assay for antibodies or immunconjugates that induce apoptosis is an annexin binding assay. In an exemplary annexin binding assay, cells are cultured

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and seeded in dishes as discussed in the preceding paragraph. The medium is removed and replaced with fresh medium alone or medium containing 0.001 to 10 μg/ml of the antibody or immunoconjugate. Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca^{2+} binding buffer, and aliquoted into tubes as discussed in the preceding paragraph. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (BD Biosciences). Antibodies or immunoconjugates that induce statistically significant levels of annexin binding relative to control are thus identified. Another exemplary assay for antibodies or immunconjugates that induce apoptosis is a histone DNA ELISA colorimetric assay for detecting internucleosomal degradation of genomic DNA. Such an assay can be performed using, e.g., the Cell Death Detection ELISA kit (Roche, Palo Alto, CA).

Cells for use in any of the above in vitro assays include cells or cell lines that naturally express STEAP-1 or that have been engineered to express STEAP-1. Such cells include tumor cells that overexpress STEAP-1 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express STEAP-1 and cell lines that do not normally express STEAP-1 but have been transfected with nucleic acid encoding STEAP-1.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit tumor growth in vivo. In vivo model systems, such as xenograft models, can be used for such testing. In an exemplary xenograft system, human tumor cells are introduced into a suitably immunocompromised non-human animal, e.g., a SCID mouse. An antibody or immunoconjugate of the invention is administered to the animal. The ability of the antibody or immunoconjugate to inhibit or decrease tumor growth is measured. In certain embodiments of the above xenograft system, the human tumor cells are tumor cells from a human patient. Such cells useful for preparing xenograft models include human prostate, lung, or colon tumor cell lines, which include without limitation PC3 cells expressing exogenous STEAP-1, and cells naturally expressing STEAP-1 which include, without limitation, LnCAP cells (Southern Research Institute, Birmingham, AL), LuCAP 77 cells, and LuCAP35V cells (University of Washington, Seattle, WA). In certain embodiments, the human tumor cells are introduced into a suitably immunocompromised non-human animal by subcutaneous injection or by transplantation into a suitable site, such as a mammary fat pad.

Binding assays and other assays

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In one aspect, an anti-STEAP-1 antibody is tested for its antigen binding activity. For example, in certain embodiments, an anti-STEAP-1 antibody is tested for its ability to bind to exogenous or endogenous STEAP-1 expressed on the surface of a cell. A FACS assay may be used for such testing.

In one aspect, competition assays may be used to identify a monoclonal antibody that competes with 120 graft or humanized variants thereof, including without limitation, 120v.24 antibody for binding to STEAP-1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear epitope peptide or a conformational epitope formed by expression of STEAP1 on a cell surface) that is bound by 120 graft antibody, or humanized 120 graft antibody, including variant 120v.24 humanized anti-STEAP-1 antibody. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). Two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more.

In an exemplary competition assay, immobilized STEAP-1 is incubated in a solution comprising a first labeled antibody that binds to STEAP-1 (e.g., murine 120.545 antibody, 120 graft antibody, or humanized 120v.24 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to STEAP-1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized STEAP-1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to STEAP-1, excess unbound antibody is removed, and the amount of label associated with immobilized STEAP-1 is measured. If the amount of label associated with immobilized STEAP-1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to STEAP-1. In certain embodiments, immobilized STEAP-1 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface.

In one aspect, purified anti-STEAP-1 antibodies can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino acid analysis, non-denaturing size exclusion high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography and papain digestion.

In one embodiment, the invention contemplates an altered antibody that possesses some but not all effector functions, which make it a desirable candidate for many applications in which the half life of the

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antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In certain embodiments, the Fc activities of the antibody are measured to ensure that only the desired properties are maintained. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991). An example of an in vitro assay to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art.

EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: Preparation of Humanized anti-STEAP-1 antibodies

Nucleic acid molecules encoding amino acid sequence variants of the antibody, antibody fragment, VL domain or VH domain are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody, antibody fragment, VL domain or VH domain. For example, libraries can be created by targeting VL accessible amino acid positions in VH, and optionally in one or more CDRs, for amino acid substitution with variant amino acids using the Kunkel method. See, for e.g., Kunkel et al., Methods Enzymol. (1987), 154:367-382 and the examples herein. Generation of randomized sequences is also described below in the Examples.

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The sequence of oligonucleotides includes one or more of the designed codon sets for a particular position in a CDR (HVR) or FR region of a polypeptide of the invention. A codon set is a set of different nucleotide triplet sequences used to encode desired variant amino acids. Codon sets can be represented using symbols to designate particular nucleotides or equimolar mixtures of nucleotides as shown in below according to the IUB code.

IUB CODES

G Guanine

A Adenine

T Thymine

C Cytosine

R (A or G)

Y (C or T)

M (A or C)

K (G or T)

S (C or G)

W (A or T)

H (A or C or T)

B (C or G or T)

V (A or C or G)

D (A or G or T)

N (A or C or G or T)

For example, in the codon set DVK, D can be nucleotides A or G or T; V can be A or G or C; and K can be G or T. This codon set can present 18 different codons and can encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys.

Oligonucleotide or primer sets can be synthesized using standard methods. A set of oligonucleotides can be synthesized, for example, by solid phase synthesis, containing sequences that represent all possible combinations of nucleotide triplets provided by the codon set and that will encode the desired group of amino acids. Synthesis of oligonucleotides with selected nucleotide "degeneracy" at certain positions is well known in that art. Such sets of nucleotides having certain codon sets can be synthesized using commercial nucleic acid synthesizers (available from, for example, Applied Biosystems, Foster City, CA), or can be obtained commercially (for example, from Life Technologies, Rockville, MD). Therefore, a set of oligonucleotides synthesized having a particular codon set will typically include a plurality of oligonucleotides with different sequences, the differences established by the codon set within

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the overall sequence. Oligonucleotides, as used according to the invention, have sequences that allow for hybridization to a variable domain nucleic acid template and also can include restriction enzyme sites for cloning purposes.

In one method, nucleic acid sequences encoding variant amino acids can be created by oligonucleotide-mediated mutagenesis. This technique is well known in the art as described by Zoller et al, 1987, Nucleic Acids Res. 10:6487-6504. Briefly, nucleic acid sequences encoding variant amino acids are created by hybridizing an oligonucleotide set encoding the desired codon sets to a DNA template, where the template is the single-stranded form of the plasmid containing a variable region nucleic acid template sequence. After hybridization, DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will contain the codon sets as provided by the oligonucleotide set.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation(s). This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al., Proc. Nat'l. Acad. Sci. USA, 75:5765 (1978).

The DNA template is generated by those vectors that are either derived from bacteriophage M13 vectors (the commercially available M13mp18 and M13mp19 vectors are suitable), or those vectors that contain a single-stranded phage origin of replication as described by Viera et al., Meth. Enzymol., 153:3 (1987). Thus, the DNA that is to be mutated can be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., above.

To alter the native DNA sequence, the oligonucleotide is hybridized to the single stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually T7 DNA polymerase or the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of gene 1, and the other strand (the original template) encodes the native, unaltered sequence of gene 1. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as E. coli JM101. After growing the cells, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabelled with a 32-Phosphate to identify the bacterial colonies that contain the mutated DNA.

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The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTT), is combined with a modified thiodeoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell.

As indicated previously the sequence of the oligonucleotide set is of sufficient length to hybridize to the template nucleic acid and may also, but does not necessarily, contain restriction sites. The DNA template can be generated by those vectors that are either derived from bacteriophage M13 vectors or vectors that contain a single-stranded phage origin of replication as described by Viera et al. ((1987) Meth. Enzymol., 153:3). Thus, the DNA that is to be mutated must be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., supra.

According to another method, a library can be generated by providing upstream and downstream oligonucleotide sets, each set having a plurality of oligonucleotides with different sequences, the different sequences established by the codon sets provided within the sequence of the oligonucleotides. The upstream and downstream oligonucleotide sets, along with a variable domain template nucleic acid sequence, can be used in a polymerase chain reaction to generate a "library" of PCR products. The PCR products can be referred to as "nucleic acid cassettes", as they can be fused with other related or unrelated nucleic acid sequences, for example, viral coat proteins and dimerization domains, using established molecular biology techniques.

Oligonucleotide sets can be used in a polymerase chain reaction using a variable domain nucleic acid template sequence as the template to create nucleic acid cassettes. The variable domain nucleic acid

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template sequence can be any portion of the heavy immunoglobulin chains containing the target nucleic acid sequences (ie., nucleic acid sequences encoding amino acids targeted for substitution). The variable region nucleic acid template sequence is a portion of a double stranded DNA molecule having a first nucleic acid strand and complementary second nucleic acid strand. The variable domain nucleic acid template sequence contains at least a portion of a variable domain and has at least one CDR. In some cases, the variable domain nucleic acid template sequence contains more than one CDR. An upstream portion and a downstream portion of the variable domain nucleic acid template sequence can be targeted for hybridization with members of an upstream oligonucleotide set and a downstream oligonucleotide set.

A first oligonucleotide of the upstream primer set can hybridize to the first nucleic acid strand and a second oligonucleotide of the downstream primer set can hybridize to the second nucleic acid strand. The oligonucleotide primers can include one or more codon sets and be designed to hybridize to a portion of the variable region nucleic acid template sequence. Use of these oligonucleotides can introduce two or more codon sets into the PCR product (i.e., the nucleic acid cassette) following PCR. The oligonucleotide primer that hybridizes to regions of the nucleic acid sequence encoding the antibody variable domain includes portions that encode CDR residues that are targeted for amino acid substitution.

The upstream and downstream oligonucleotide sets can also be synthesized to include restriction sites within the oligonucleotide sequence. These restriction sites can facilitate the insertion of the nucleic acid cassettes (i.e., PCR reaction products) into an expression vector having additional antibody sequence. In one embodiment, the restriction sites are designed to facilitate the cloning of the nucleic acid cassettes without introducing extraneous nucleic acid sequences or removing original CDR or framework nucleic acid sequences.

Nucleic acid cassettes can be cloned into any suitable vector for expression of a portion or the entire light or heavy chain sequence containing the targeted amino acid substitutions generated via the PCR reaction. According to methods detailed in the invention, the nucleic acid cassette is cloned into a vector allowing production of a portion or the entire light or heavy chain sequence fused to all or a portion of a viral coat protein (i.e., creating a fusion protein) and displayed on the surface of a particle or cell. While several types of vectors are available and may be used to practice this invention, phagemid vectors are the preferred vectors for use herein, as they may be constructed with relative ease, and can be readily amplified. Phagemid vectors generally contain a variety of components including promoters, signal sequences, phenotypic selection genes, origin of replication sites, and other necessary components as are known to those of ordinary skill in the art.

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When a particular variant amino acid combination is to be expressed, the nucleic acid cassette contains a sequence that is able to encode all or a portion of the heavy or light chain variable domain, and is able to encode the variant amino acid combinations. For production of antibodies containing these variant amino acids or combinations of variant amino acids, as in a library, the nucleic acid cassettes can be inserted into an expression vector containing additional antibody sequence, for example all or portions of the variable or constant domains of the light and heavy chain variable regions. These additional antibody sequences can also be fused to other nucleic acids sequences, such as sequences that encode viral coat proteins and therefore allow production of a fusion protein.

Humanization of murine anti-human STEAP-1 antibody is described herein.

Materials and Methods

Residue numbers are according to Kabat (Kabat *et al., Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Single letter amino acid abbreviations are used. DNA degeneracies are represented using the IUB code (N = A/C/G/T, D = A/G/T, V = A/C/G, B= C/G/T, H= A/C/T, K = G/T, M = A/C, R = A/G, S = G/C, W= A/T, Y = C/T).

Cloning of murine 120 variable domains and generation of a chimeric 120 antibody – Total RNA was extracted from hybridoma cells producing M2-120.545 (designated as "murine 120" or "mu120" herein) using standard methods. The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers to the heavy and light chains. The forward primers were specific for the N-terminal amino acid sequence of the VL and VH regions. Respectively, the LC and HC reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which are highly conserved across species. Amplified VL and VH were cloned into mammalian expression vectors. The polynucleotide sequence of the inserts was determined using routine sequencing methods. The M2-120.545 ("mu 120") VL and VH amino acid sequences are shown in Figures 2A and 2B, respectively.

Generation of murine 120 chimera – A chimeric anti-STEAP-1 antibody was prepared by fusing the murine 120 variable heavy (VH) and variable light (VL) regions to the constant domains of a human IgG. The resultant antibody is designated "120 chimera," "chimera 120," "chimeric 120 IgG," or "Fc chimera" herein.

Direct hypervariable region grafts onto the acceptor human consensus framework – Variants constructed during the humanization of murine 120 were assessed both as protein in the form of an IgG or as a Fab displayed on phage.

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The phagemid used for this work is a monovalent Fab-g3 display vector and consists of two open reading frames under control of the phoA promoter. The first open reading frame consists of the stII signal sequence fused to the VL and CH1 domains of the acceptor light chain and the second consists of the stII signal sequence fused to the VH and CH1 domains of the acceptor heavy chain followed by the minor phage coat protein P3.

The VL and VH domains from murine 120 were aligned with the human VL kappa I (huKI) and human VH subgroup III (huIII) consensus sequences. To make the CDR grafts, hypervariable regions from the murine 120 antibody were grafted into the huKI and huIII acceptor frameworks.

Hypervariable regions from murine 120 antibody (mu120) were engineered into the acceptor human consensus framework to generate the direct CDR-graft (designated as "120 graft," or "graft 120" herein). In the VL domain the following regions were grafted to the human consensus acceptor: positions 24-34 (L1), 50-56 (L2) and 89-97 (L3). In the VH domain, positions 26-35a (H1), 49-65 (H2) and 95-102 (H3) were grafted. The sequences of the light and heavy chain variable regions of the 120 graft are shown in Figures 2A-2B. The CDRs (also designated here as HVRs) are shown in boxes (Figures 2A-2B). These CDR definitions include positions defined by their sequence hypervariability (Kabat ref), their structural location (Chothia ref) and their involvement in antigen-antibody contacts (MacCallum *et al. J. Mol. Biol.* 262: 732-745 (1996)).

The direct-graft variants expressed as a Fab displayed on phage or as an IgG were generated by Kunkel mutagenesis using a separate oligonucleotide for each hypervariable region. Correct clones were assessed by DNA sequencing.

Generation of humanized 120 phage variants – Humanized 120 variants were generated as Fab displayed on phage by Kunkle mutagenesis. A phosphorylated oligonucleotide was added to 300 ng Kunkel template in 50 mM Tris pH 7.5, 10 mM $MgCl_2$ in a final volume of 10 µl. The mixture was annealed at 90 °C for 2 min, 50 °C for 5 min and then cooled on ice. The annealed template was then filled in by adding 0.5 µl 10mM ATP, 0.5 µl 10mM dNTPs (10mM each of dATP, dCTP, dGTP and dTTP), 1 µl 100mM DTT, 1 µl 10X TM buffer (0.5 M Tris pH 7.5, 0.1 M $MgCl_2$), 80 U T4 ligase, and 4 U T7 polymerase in a total volume of 20 µl for 2 h at room temperature. The filled in and ligated product was then transformed into XL1-blue cells (Stratagene). Correct clones were identified by DNA sequencing.

Correct phage clones were grown in 25 ml of 2YT containing 50 µg/ml carbenacillin and M13/KO7 helper phage (MOI 10) overnight at 37 °C.

Assessment of humanized 120 variants – Humanized variants expressed as IgG were assessed by FACS analysis using Steap1 positive (293 Steap1 NT LB50) and negative (293 vector S408) cell lines.

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Humanized variants expressed as a Fab displayed on phage were also assessed by FACS analysis. Phage expressing Fab variants were first assessed for their Fab display level using an phage ELISA used to detect a flag-tag fused to the light chain of the Fab. MaxiSorp microtiter plates were coated with anti-gD 1766 at 10 μg/ml in PBS over night and then blocked with Casein Blocker. Phage from culture supernatants were serially diluted in PBST containing 0.5 % BSA in a tissue culture microtiter plate and transferred to the coated wells for 1 h to capture the Fab displaying phage. The plate was washed with PBST and HRP conjugated anti-M13 (Amersham Pharmacia Biotech) was added (1:5000 in PBST containing 0.5 % BSA) for 40 min. The plate was washed with PBST and developed by adding Tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The absorbance at 405 nm was used as an estimate of the Fab display level on the surface of the phage. Phage preparations were normalized for display by dilution. Low displaying phage (e.g. the chimera) were used neat for FACS analysis.

For FACS analysis of phage binding, cells were removed from the plate using 2 mM EDTA, collected in a 15 mL conical bottom tube and pelleted by centrifugation. Cells (5 X 10^5 cells per sample) were re-suspended in 100 μL of phage (normalized by display level) in FACS buffer (1% FBS, PBS with 2 mM EDTA) and incubated for 1-2 hours on ice. Samples were washed twice with FACS buffer by centrifugation. Anti-M13 5G7 control antibody (Genentech, Inc. South San Francisco, CA) was added at 2 μg/mL and incubated on ice for at least 45 minutes. Samples were washed twice with FACS buffer by centrifugation. A 1:200 dilution of anti-mouse PE (R-phycoerythrin goat anti-mouse IgG Fcy Fragment, Jackson Immunoresearch) was added and incubated on ice for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and analyzed by FACS.

For analysis of IgG by FACS, cells were prepared as in the phage FACS. Each IgG was added at 5 μg/mL on ice for 1 hour. Samples were washed twice with FACS buffer by centrifugation and a 1:200 dilution of anti-human PE conjugate (R-phycoerythrin goat anti-human IgG Fcy Fragment, Jackson Immunoresearch) was added for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and samples were analyzed by FACS.

IgG Production and Affinity Determination -IgG was purified with Protein G affinity chromatography. Affinity determinations were performed by Scatchard analysis on 293 STEAP-1 NT LB50 cells.

Results and Discussion

Murine 120 variable domain sequences and CDR assignment graft design – The human acceptor framework used for humanization of M2-120.545 is based on the consensus human kappa I VL domain and the human subgroup III consensus VH domain. The VL and VH domains of murine M2-120.545 were each aligned with the human kappa I and subgroup III domains; each complimentarity region (CDR) was

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identified and grafted into the human acceptor framework to generate a CDR graft that could be displayed as an Fab on phage and expressed as an IgG. The sequences of humanized anti-STEAP-1 antibody version 24 variable regions are shown in Figures 2A and 2B. The 120-graft Fab displayed on phage and 120-graft IgG were tested for binding to exogenous STEAP-1 expressing cells (293 STEAP-1 NT LB50) by FACS analysis. Although the 120-graft IgG bound specifically to the STEAP-1 expressing cells, the FACS signal observed for the 120-graft IgG was smaller than that observed for the chimeric 120 IgG indicating a loss in binding affinity. Phage displaying the 120-graft Fab also generated a FACS signal that was only observed on STEAP-1 expressing cells. This shift was less than that observed for the chimeric 120 IgG. Scatchard analysis of the 120-graft IgG also indicated a significant (approximately 50-fold) loss in binding affinity (KD = 36 nM for the 120v.78; KD = 260 nM for 120 graft).

Humanization of M2-120.545 –Approximately 30 vernier positions that influence CDR conformation and VL:VH domain packing have been identified and changes at these positions between the donor and human frameworks should be considered when humanizing antibodies (Foote, J. and Winter, G., J. Mol. Biol.224(2):487-499 (1992)). An assessment of the alignment of murine M2-120.545 with the consensus human kappa I VL domain and the human subgroup III consensus VH domain revealed sequence differences at 6 key vernier positions in the VH domain: 24, 37, 48, 67, 73, 78 (see Figure 2B). To assess the influence of these positions, murine residues were individually introduced into the human consensus subgroup III VH domain of the Fab on phage. This involved making the following mutations to the 120-graft Fab displayed on phage individually: A24V (120.v24), V37I (120.v37), V48M (120.v48), F67I (120.v67), and L78F (120.v78). N73T was not tested. Each phage variant was normalized by dilution to an equivalent Fab display level determined by titration of an epitope tag fused to the light chain displayed on the phage and then assessed for binding to STEAP-1 by FACS analysis on STEAP-1-expressing cells (293 STEAP-1 NT LB50) and non-expressing (293 vector S408) cells. The term "2°" refers to the secondary antibody in the FACS analysis. The term "α-120" refers to the murine 120 anti-STEAP-1 antibody. The term "α-10H1" refers to a control antibody. The terms "24 Phage," "37 Phage," and the like refer to humanized anti-STEAP-1 variants as disclosed herein displayed on phage. "Ch 120 Phage" refers to the 120 Chimera displayed on phage, and "120.graft Phage" refers to the 120 graft displayed on Phage. (Figure 6). The importance of normalizing phage clones by their Fab display level is illustrated by a FACS analysis of the 120-graft at different phage titers: $7x10^{12}$ phage/ml in Figure 6 and 2×10^{11} phage/ml in Figure 6. Once diluted to the lower phage concentration, the 120-graft phage no longer produced an observable FACS shift. Thus normalization of the different phage clones for their display level was important step for assessing their affinity differences for Steap1.

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Following normalization for Fab display levels, the variant of the 120-graft containing the additional mutation A24V (120.v24) produced a FACS shift superior to other variants (Figure 6). When expressed as an IgG, 120.v24 produced a similar FACS shift to the chimeric 120 antibody at all concentrations tested. Subsequent Scatchard analysis of 120.v24 indicated a Kd of 2.2 nM for binding to 293 STEAP-1 NT LB50 cells, a two-fold improvement over the 120 chimera and the original murine M2-120.545 (Table 2).

Table 2: Anti-STEAP-1 antibody binding affinity for cell surface STEAP-1 (Kd (nM))

Cell line	Murine anti-STEAP-1 MAb 120.545 nM	120 Chimera	Humanized anti-STEAP-1 120v.24
PC3-PS5.4 (exogenous STEAP-1)	17.5 nM 187,256 sites per cell	9.9 nM 103,204 sites per cell	---
293.LB50 (exogenous STEAP-1)	4.7 nM 301,100 sites per cell	4.9 nM 252,892 sites per cell	2.2 nM 264,172 sites per cell
LNCaP-BR (endogenous STEAP-1)	1.5 nM 37,207 sites per cell	0.9 nM 22,021 sites per cell	---

Binding activity of anti-STEAP-1 naked antibodies, murine 120 and chimera 120 was also tested using FACS analysis. Binding was compared for exogenous STEAP-1 in 293 stable STEAP-1 NT LB50, PC3 stable STEAP-1 PS5.4, and endogenous STEAP-1 in LNCaP cells. The results are also shown in Figs. 7D-7F. NT LB50 cells expressing exogenous human STEAP-1 on the cell surface were prepared by stably transforming 293 cells (ATCC CRL-1573) with human STEAP-1 DNA. PS5.4 cells expressing exogenous human STEAP-1 on the cell surface were prepared by stably transforming PC3 (ATCC CLL-1435) with human STEAP-1 DNA. LNCaP cells (ATCC CRL-1740) express STEAP-1 endogenously.

Example 2: Characterization of anti-STEAP-1 antibodies

Anti-STEAP-1 antibodies (naked antibodies and antibody drug conjugates disclosed herein) were characterized or may be characterized according to standard methods.

ELISA-based assays: Anti-STEAP-1 antibody screening by ELISA is performed as follows, with all incubations done at room temperature. Test plates (Nunc Immunoplate) were coated for 2 hours with

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purified STEAP-1 in 50 mM sodium carbonate buffer, pH 9.6, then blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS) for 30 minutes, then washed four times with PBS containing 0.05% Tween 20 (PBST). Test antibody supernatants are added and incubated two hours with shaking, then washed four times with PBST. The plates are developed by adding 100 µl/well of a solution containing 10 mg of o-phenylenediamine dihydrochloride (Sigma, #P8287) and 10 µl of a 30% hydrogen peroxide solution in 25 ml phosphate citrate buffer, pH 5.0, and incubating for 15 minutes. The reaction is stopped by adding 100 µl/well of 2.5 M sulfuric acid. Data is obtained by reading the plates in an automated ELISA plate reader at an absorbance of 490 nm.

Characterization of anti-STEAP-1 binding by Scatchard analysis:

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980) using standard techniques well know in the relevant art. See also Scathcard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

Example 3: Production of Anti-STEAP-1 Antibody Drug Conjugates

Production of anti-STEAP-1 auristatin ADCs - Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies murine 120.545, 120 chimera, 120 graft, and humanized 120 framework variants to the following drug-linker moieties: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE, MC-MMAF, vc-MMAE, and vc-MMAF, which drug and linker moieties and methods of attachment are disclosed herein as well as in WO 2004/010957, published February 5, 2004, WO2006/034488, published September 9, 2005, and in Doronina, S.O. et al., Nature Biotechnol. 21:778-784 (2003), (each of which references is herein incorporated by reference in its entirety). Prior to conjugation, the antibodies were partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957. The partially reduced antibodies were conjugated to the above drug-linker moieties using standard methods in accordance with the methodology described in Doronina et al. (2003) Nat. Biotechnol. 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibodies were combined with the drug linker moieties to allow conjugation of the moieties to cysteine residues. The conjugation reactions were quenched, and the ADCs were purified. The drug load (average number of drug moieties per antibody) for each ADC was determined by HPLC. As used herein, the linker-drug component of an ADC, " –MC-vc-PAB-MMAE" or "-MC-vc-PAB-MMAF" is sometimes abbreviated as " –vcMMAE" or "-vcMMAF," and the component "-MC-MMAF" is sometimes abbreviated as "MCMMAF" or "mcMMAF."

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Production of anti-STEAP-1 maytansinoid ADCs – Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies, murine 120, 120 chimera, 120 graft, and humanized 120 framework variants to the linker drug moiety –smcc-DM1. Such conjugation may be performed according to the method disclosed in WO 2005/037992 for conjugation of Herceptin® anti-HER2 antibody.

Example 4: In Vivo Tumor Volume Reduction Assay

To test the efficacy of toxin-conjugated or unconjugated anti-STEAP-1 monoclonal antibodies for the ability to reduce tumor volume in vivo and in vitro, the following protocol was employed.

Mammalian cell lines and human tumor xenografts: 293 is a human immortalized embryonic kidney cell line (ATCC reference CRL1573), PC-3 is a human prostate adenocarcinoma cell line (ATCC reference CRL1435) and LNCaP is a prostate carcinoma cell line (ATCC CRL1740). All cells were grown in 50/50 Dulbecco modified Eagle high glucose medium, Ham's F12 supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, 1% penicillin-streptomycin and cultured at 37°C in 5% CO2. 293 and PC-3 stable cell lines were generated by transfection (Fugene, Roche) with a cytomegalovirus-driven vector encoding either full length STEAP1 (LB50 and PS5.4 respectively) or an empty vector and selected in 400 µg/ml G418 (Geneticin, Life Technologies). Human prostate explant models, LuCAP 77 and LuCAP 35V, were obtained from the University of Seattle.

Expression of exogenous and endogenous STEAP-1 on the cell surface was demonstrated by immunohistohemistry (IHC) and FACS analysis as follows. Sheep and mouse anti-STEAP-1 antibodies (Agensys, Inc., Santa Monica, CA) were generated against an intracellular amino-terminal peptide of STEAP-1 (see Hubert, R.S., Vivanco, I. et al., PNAS 25:14523-14528 (1999)). Monoclonal antibodies against the extracellular domains of STEAP-1 (Agensys, Inc.) were generated by immunization of mice with 293T cells transiently transfected with STEAP-1. For IHC analysis, the primary sheep anti-STEAP-1 antibody was used for detection. For FACS analysis, cells were grown to 90% confluence and removed from plates using 2 mM EDTA in PBS. Cells were washed and resuspended in FACS buffer (PBS with 1% BSA) and incubated for 60 minutes with anti-STEAP1 antibodies at room temperature followed by 60 minutes with the appropriate secondary antibody conjugated to phycoerythrin. Analysis was performed on FACSscan (BD Biosciences). For immunoflourescence, cells were grown in chamber slides overnight and then incubated with primary antibody at 37°C for 60 minutes. Cells were fixed in paraformaldehyde , blocked in 1% BSA and incubated with the appropriate secondary antibody conjugated to flourescin.

In vivo prostate cancer xenograft models were used to test the efficacy of anti-STEAP-1 ADCs. These models included human cell line LNCaP (ATCC CRL-1740 or Southern Research Institute,

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Birmingham, AL). Prostate explant models included LuCaP 77 and LuCaP35V (University of Washington, Seattle, WA). Each prostate explant model was maintained by serial transplanting in castrated (androgen independent model, LuCAP 35V) or uncastrated (androgen dependent model, LuCAP 77), male SCID-beige mice from Charles River Lab. The uncastrated mice received a testosterone pellet prior to implantation, while castration was done at least two weeks prior to tumor implantation to allow testosterone levels to nadir. When donor mice had tumors of between 800-1000 mm^3, tumor tissue were aseptically removed and dissected into small implantable sized pieces (approximately 20 mm^3) for study animals. The tumor is placed into a pocket at the implantation site and the skin is closed using wound clips. For the LNCaP cell line model, in vitro grown LNCaP cells were injected subcutaneously at 8-10 million cells per mouse in 50% matrigel into male SCID-beige mice which had received a testosterone pellet. When mean tumor size reached 100-200mm^3, animals were randomly grouped in ten groups of ten mice each and given a single IV administration of test antibody ADC or control antibody (naked or control). In some experiments, multiple doses of test or control antibody were administered (see Figures 8A, 9, and 10). In some experiments, a single dose of test and control antibody were administered as seen in Figures 8B and 11. Where the prostate explant model was LuCap 77, a testosterone pellet was implanted in the mice approximately 3-7 days before transplantation of exogenous tumor. Tumors were measured twice per week for 4 weeks, then once or twice per week for the remainder of the study or once per week throughout the study. A significantly lower tumor volume in test animals over time was considered to an indication of efficacy. In some cases, tumor volume decreased significantly from the initial volume and remained low throughout the study. Results are plotted in Figures 8-11.

Anti-STEAP-1 auristatin drug conjugates reduce prostate tumor volume in vivo

Administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. PBS and anti-gp120-MC-vc-PAB-MMAE (3 mg/kg) were used as controls. Doses were administered on days 0, 7, and 14. See Figure 8A.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg), and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) to SCID beige mice transplanted with LNCap-Ner tumor (treated with a testosterone pellet as described herein) was shown to be efficacious. Vehicle, anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (12 mg/kg) were used as controls. Doses were administered on the days indicated in Figure 8. The results are plotted in Figure 8B.

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Administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) and anti-STEAP-1 120 chimera-MC-MMAF (6 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID-beige mice transplanted with LNCaP cells. Three doses at approximately days 15, 25, and 30 at 3 mg/kg (anti-STEAP-vcMMAE) or 6 mg/kg (anti-STEAP-mcMMAF) were administered to the mice. Control anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (6 mg/kg) were used. See Figure 9.

Administration of humanized anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. Controls were vehicle and anti-ragweed-MC-vc-PAB-MMAE. Three doses at 3 mg/kg of test and control antibodies were administered. See Figure 10.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. Drug load was 3.1 per antibody. Control antibodies were anti-ragweed-MC-MMAF administered at 12 mg/kg, and anti-gp120-MC-vc-PAB-MMAE administered at 6 mg/kg. See Figure 11.

Anti-STEAP-1 auristatin drug conjugates reduce prostate tumor volume in vitro

In vitro cell killing assays were performed to assess the effectiveness of anti-STEAP-1 drug conjugates to inhibit growth and/or kill cells expressing STEAP-1. Briefly, cells expressing STEAP-1 were plated at approximately 2,000 cells/well in a 96-well plate and treated 24 hours later in duplicate with antibody drug conjugate. Plates were incubated for 5-7 days at 37°C and developed with CellTiter-Glo® luminescent cell viability assay kit (Promega, Madison, WI, USA). Test cells included PS5.4 (PC3 cells expressing exogenous STEAP-1), LB50 (293 cells expressing exogenous STEAP-1), PC3 cells transfected with vector alone, 293 cells transfected with vector alone, and LNCaP cells expressing endogenous STEAP-1. Tested antibody drug conjugates included control antibody-MC-MMAF, control antibody-vc-MMAE, anti-STEAP-1 antibody 120 chimera-vc-MMAE, anti-STEAP-1 antibody 120 chimera-MC-MMAF (two different lots of material), and anti-STEAP-1 antibody chimera-vc-MMAF. The results are shown in Figure 14A-E.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

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WHAT IS CLAIMED IS:

1. An antibody that binds to STEAP-1, wherein the antibody comprises at least one, two, or three heavy chain (HC) hypervariable regions (HVRs) selected from:
(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

2. The antibody of claim 1, further comprising comprising at least one, two, or three HC framework regions (FRs) selected from:
(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

3. The antibody of claim 1, further comprising a light chain (LC) of SEQ ID NO:6.

4. The antibody of claim 2, further comprising a light chain (LC) of SEQ ID NO:6.

5. The antibody of claim 1, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

6. The antibody of claim 2, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

7. The antibody of claim 1, wherein the antibody is humanized.

8. The antibody of claim 2, wherein the antibody is humanized.

9. The antibody of claim 1, wherein the STEAP-1 is mammalian STEAP-1.

10. The antibody of claim 9, wherein the STEAP-1 is selected from rodent STEAP-1 and primate STEAP-1.

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11. The antibody of claim 31, wherein the STEAP-1 is human STEAP-1.

12. A polynucleotide encoding a HC comprising at least one, two, or three HC-HVRs selected from:
(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

13. A polynucleotide of claim 12 comprising at least one, two, three, or four HC-FRs selected from:
(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

14. A vector comprising the polynucleotide of claim 12.

15. A host cell comprising the vector of claim 12.

16. The host cell of claim 15, wherein the host cell is a bacteria, yeast, insect, or eukaryotic cell.

17. The host cell of claim 42, wherein the host cell is a CHO cell.

18. A method of making an anti-STEAP-1 antibody, wherein the method comprises a) culturing the host cell of claim 15 under conditions suitable for expression of the polynucleotide encoding the antibody, and b) isolating the antibody.

19. The antibody of claim 1, wherein the STEAP-1 is expressed on the surface of a cell.

20. The antibody of claim 19, wherein the cell is a prostate, lung, colon, bladder, or ovary cell or a Ewing's sarcoma cell.

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21. The antibody of claim 20, wherein the cell is associated with a proliferative disorder.

22. The antibody of claim 21, wherein the disorder is cancer.

23. The antibody of claim 22, wherein the cancer is metastasis.

24. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

25. The antibody of claim 24, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

26. The antibody of claim 60, wherein the antibody is humanized.

27. The antibody of claim 60, wherein the antibody is human.

28. A method of detecting the presence of STEAP-1 in a biological sample, the method comprising contacting the biological sample with an antibody of claim 1 under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1.

29. The method of claim 28, wherein the biological sample is from a patient suspected of having a prostate, lung, colon, bladder, ovarian cell or Ewing's sarcoma cell proliferative disorder.

30. An immunoconjugate comprising an antibody of claim 1 covalently attached to a cytotoxic agent.

31. The immunoconjugate of claim 30, wherein the cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

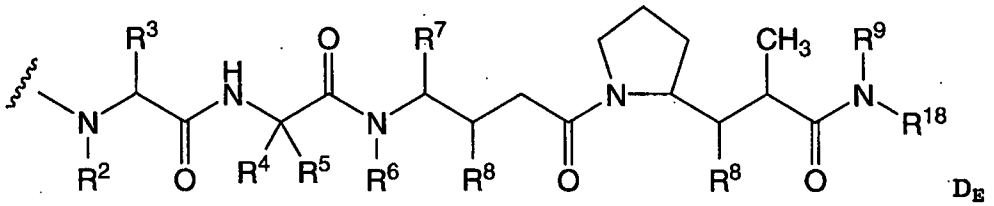
32. The immunoconjugate of claim 31, the immunoconjugate having the formula Ab-(L-D)p, wherein:
    (a) Ab is the antibody of claim 1;
    (b) L is a linker;
    (c) D is a drug moiety.

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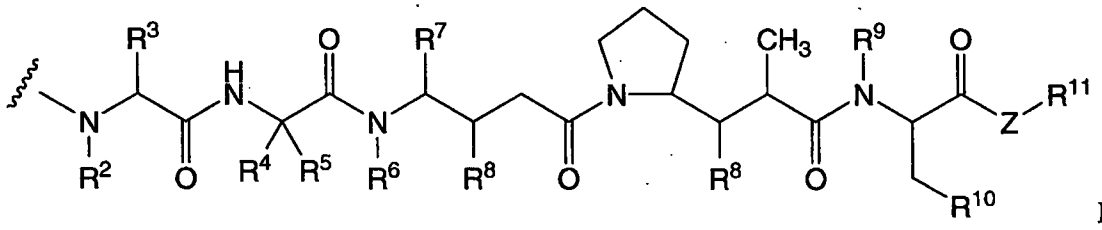
33. The immunoconjugate of claim 32, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenyloxycarbonyl (MC-vc-PAB).

34. The immunoconjugate of claim 33, wherein D is selected from an auristatin and dolostantin.

35. The immunoconjugate of claim 34, wherein D is a drug moiety of formula D_E or D_F:



D_E



D_F

and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-CH_3$; and R^{18} is $-C(R^8)_2-C(R^8)_2-$aryl; and

(d) p ranges from about 1 to 8.

36. The immunoconjugate of claim 32, having in vitro or in vivo cell killing activity.

37. The immunoconjugate of claim 32, wherein the linker is attached to the antibody through a thiol group on the antibody.

38. The immunoconjugate of claim 32, wherein the linker is cleavable by a protease.

39. The immunoconjugate of claim 32, wherein the linker comprises a val-cit dipeptide.

40. The immunoconjugate of claim 32, wherein the linker comprises a p-aminobenzyl unit.

41. The immunoconjugate of claim 32, wherein the linker comprises 6-maleimidocaproyl.

42. The immunoconjugate of claim 32, wherein the drug is selected from MMAE and MMAF.

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43. The immunoconjugate of claim 42, wherein the drug is MMAE.

44. The immunoconjugate of claim 42, wherein the drug is MMAF.

45. The immunoconjugate of claim 32, wherein the immunoconjugate has the formula

Ab-(L-MMAE)p,

wherein L is a linker and p ranges from 2 to 5.

46. The immunoconjugate of claim 45, wherein L comprises val-cit.

47. The immunoconjugate of claim 45, wherein L comprises MC.

48. The immunoconjugate of claim 45, wherein L comprises PAB.

49. The immunoconjugate of claim 45, wherein L comprises MC-vc-PAB.

50. The immunoconjugate of claim 45, wherein the immunoconjugate has the formula

Ab-(L-MMAF)p,

wherein L is a linker and p ranges from 2 to 5.

51. The immunoconjugate of claim 50, wherein L comprises val-cit.

52. The immunoconjugate of claim 50, wherein L comprises MC.

53. The immunoconjugate of claim 50, wherein L comprises PAB.

54. The immunoconjugate of claim 50, wherein L comprises MC-vc-PAB.

55. The immunoconjugate of claim 50, wherein the immunoconjugate has the formula

Ab-(L-MMAF)p,

wherein L is a linker and p ranges from 2 to 5.

56. The immunoconjugate of claim 32, wherein D is a maytansinoid.

57. The immunoconjugate of claim 56, wherein D is selected from DM1, DM3, and DM4.

58. The immunoconjugate of claim 56, having in vitro or in vivo cell killing activity.

59. The immunoconjugate of claim 56, wherein the linker is attached to the antibody through a thiol group on the antibody.

60. The immunoconjugate of claim 56, wherein the linker, L, is selected from N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

61. The immunoconjugate of claim 60, wherein the drug is DM1.

62. The immunoconjugate of claim 62, wherein L comprises a SPP.

63. The immunoconjugate of claim 61, wherein L comprises SMCC.

64. The immunoconjugate of claim 61, wherein p is from 2-6.

65. The immunoconjugate of claim 64, wherein p is from 3-5.

66. A pharmaceutical composition comprising the immunoconjugate of claim 32 and a pharmaceutically acceptable carrier.

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67. A method of treating a cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 66.

68. The method of claim 67, wherein the cell proliferative disorder is selected from prostate, lung, colon, bladder, ovary, and Ewing's sarcoma cell proliferative disorder.

69. A method of inhibiting prostate, lung, colon, bladder, ovary, Ewing's sarcoma cell proliferation comprising exposing a cell to an immunoconjugate of claim 32 under conditions permissive for binding of the immunoconjugate to STEAP-1.

70. The method of claim 69, wherein the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell is a xenograft.

71. The method of claim 69, wherein the exposing takes place in vitro.

72. The method of claim 69, wherein the exposing takes place in vivo.

73. The antibody of claim 1, wherein the antibody is a cysteine engineered antibody comprising one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0, wherein the cysteine engineered antibody is prepared by a process comprising replacing one or more amino acid residues of a parent antibody by cysteine.

74. The cysteine engineered antibody of claim 73, wherein the cysteine engineered antibody is more reactive than the parent antibody with a thio-reactive reagent.

75. The cysteine engineered antibody of claim 73 wherein the process further comprises determining the thiol reactivity of the cysteine engineered antibody by reacting the cysteine engineered antibody with a thiol-reactive reagent;

wherein the cysteine engineered antibody is more reactive than the parent antibody with the thiol-reactive reagent.

76. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a light chain.

77. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a heavy chain.

78. The cysteine engineered antibody of claim 73, wherein the antibody is an immunoconjugate comprising the cysteine engineered antibody covalently attached to a cytotoxic agent.

79. The cysteine engineered antibody of claim 78, wherein the cytotoxic agent is selected fom a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

80. The cysteine engineered antibody of claim 73 wherein the antibody is covalently attached to a capture label, a detection label, or a solid support.

81. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a biotin capture label.

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82. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a fluorescent dye detection label.

83. The cysteine engineered antibody of claim 82 wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

84. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a radionuclide detection label selected from ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi.

85. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a chelating ligand that chelates a detectable label.

86. The cysteine engineered antibody of claim 80 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

87. The immunoconjugate of claim 32, wherein the linker is attached to the antbody through a thiol group on the antibody.

88. The antibody of claim 1 comprising an albumin binding peptide.

89. The antibody of claim 88, wherein the albumin binding peptide is selected from SEQ ID NOs:80-84.

90. The method of claim 67, further comprising administering to the individual an effective amount of at least one additional therapeutic compound selected from a chemotherapeutic acid, a radionuclide, CHOP, a naked antibody, antibody drug conjugate, and an immunoconjugate.

91. The method of claim 90, wherein the antibody, ADC, and immunoconjugate bind to an antigen expressed in proliferating cells.

92. The method of claim 91, wherein the proliferating cells are from prostate, lung, colon, bladder, ovary, or Ewing's sarcoma.

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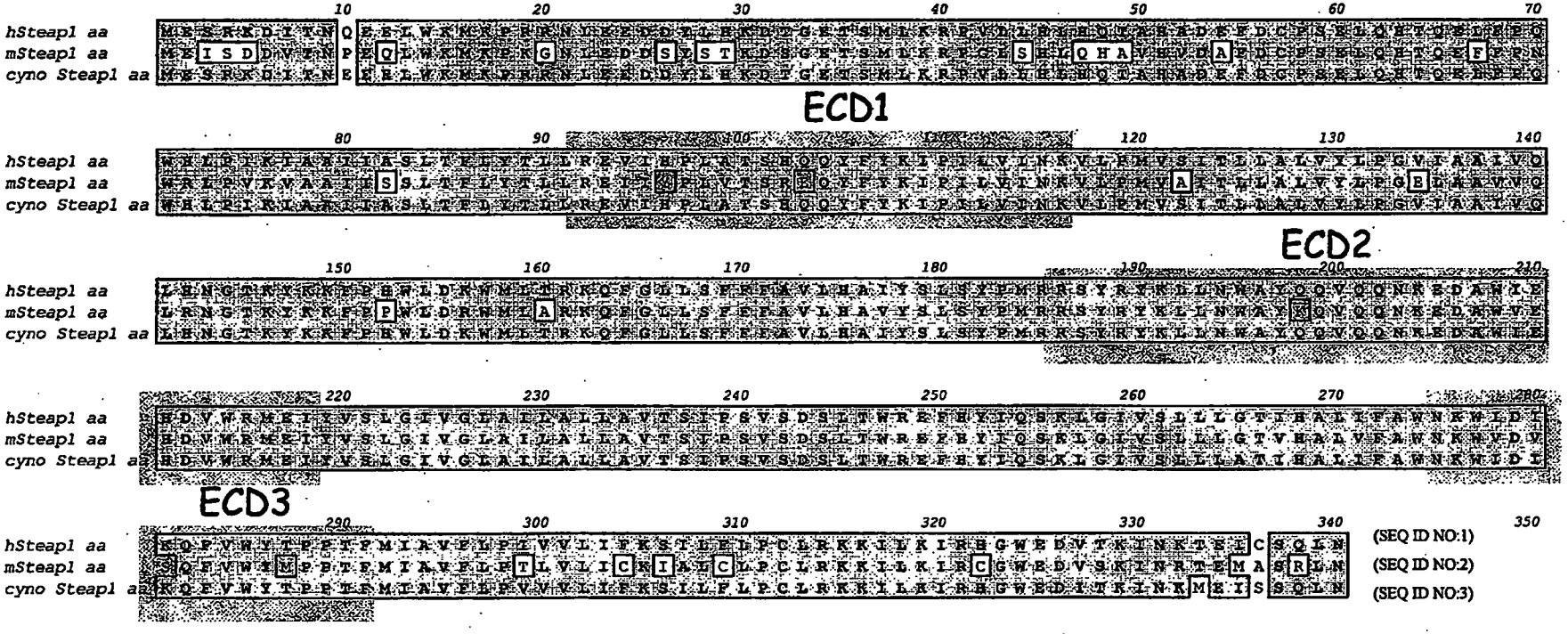
ABSTRACT OF THE DISCLOSURE

Anti-STEAP-1 antibodies and immunoconjugates thereof are provided. Methods of using anti-STEAP-1 antibodies and immunoconjugates thereof are provided.

5

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Figure 1



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Alignment of Light Chain Sequences

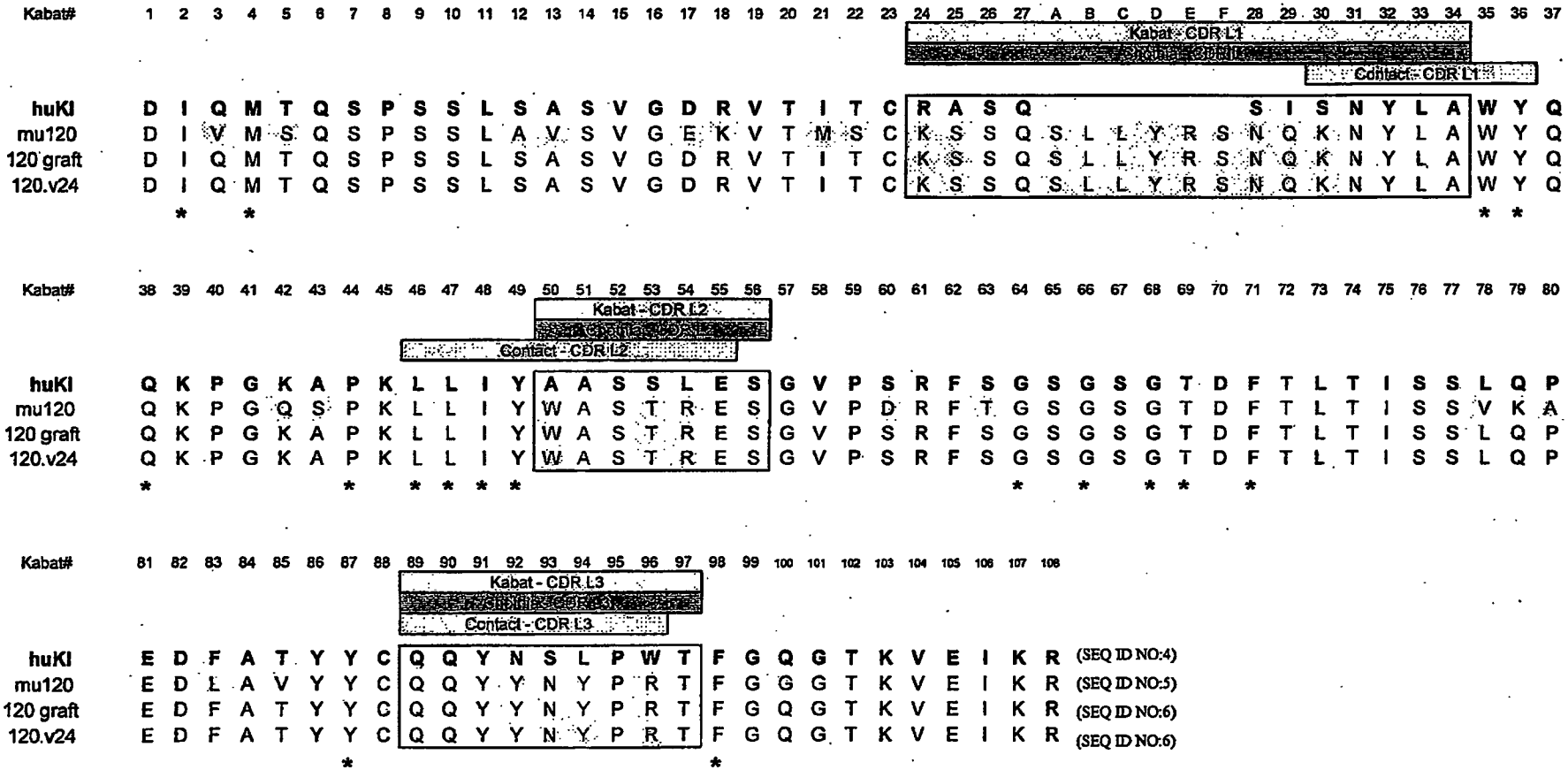


Figure 2A

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Alignment of Heavy Chain Sequences

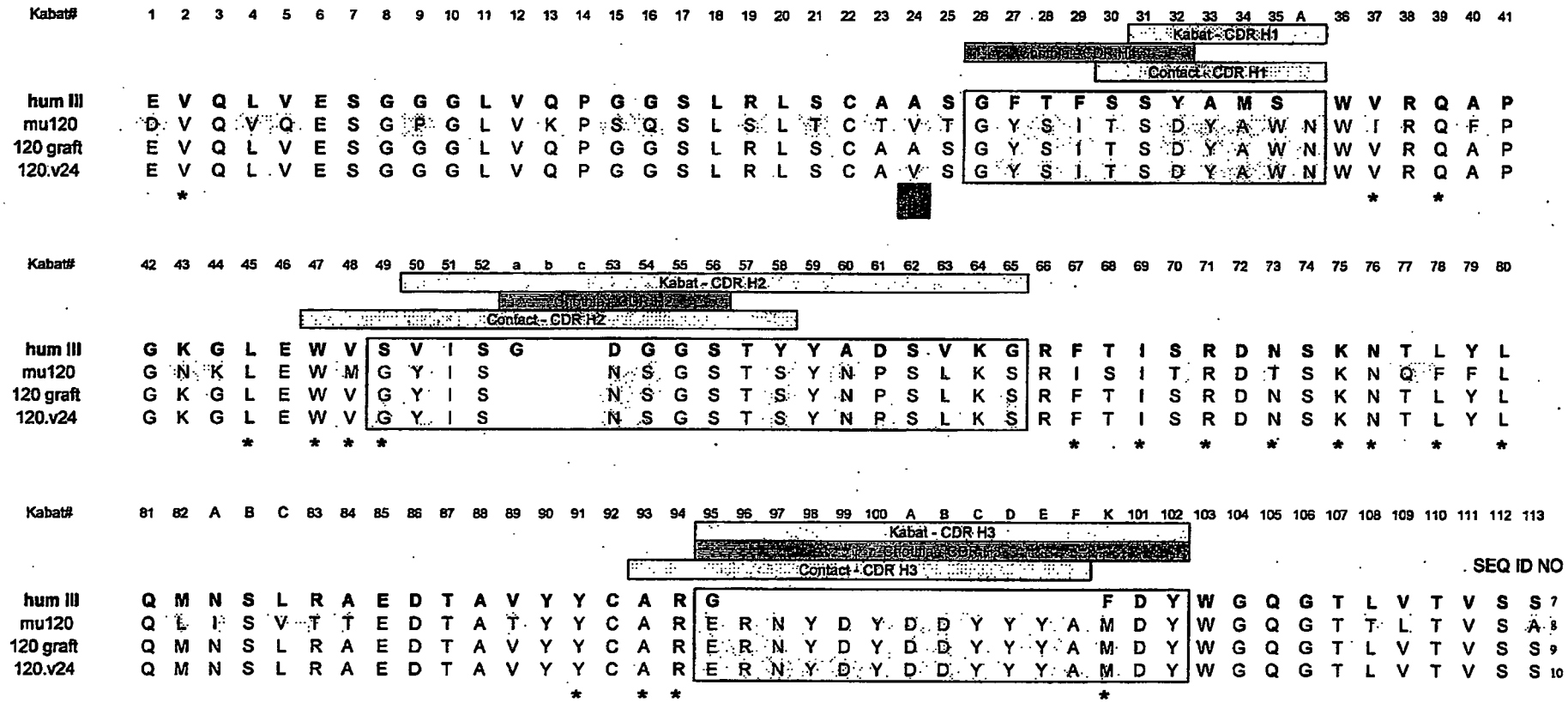


Figure 2B

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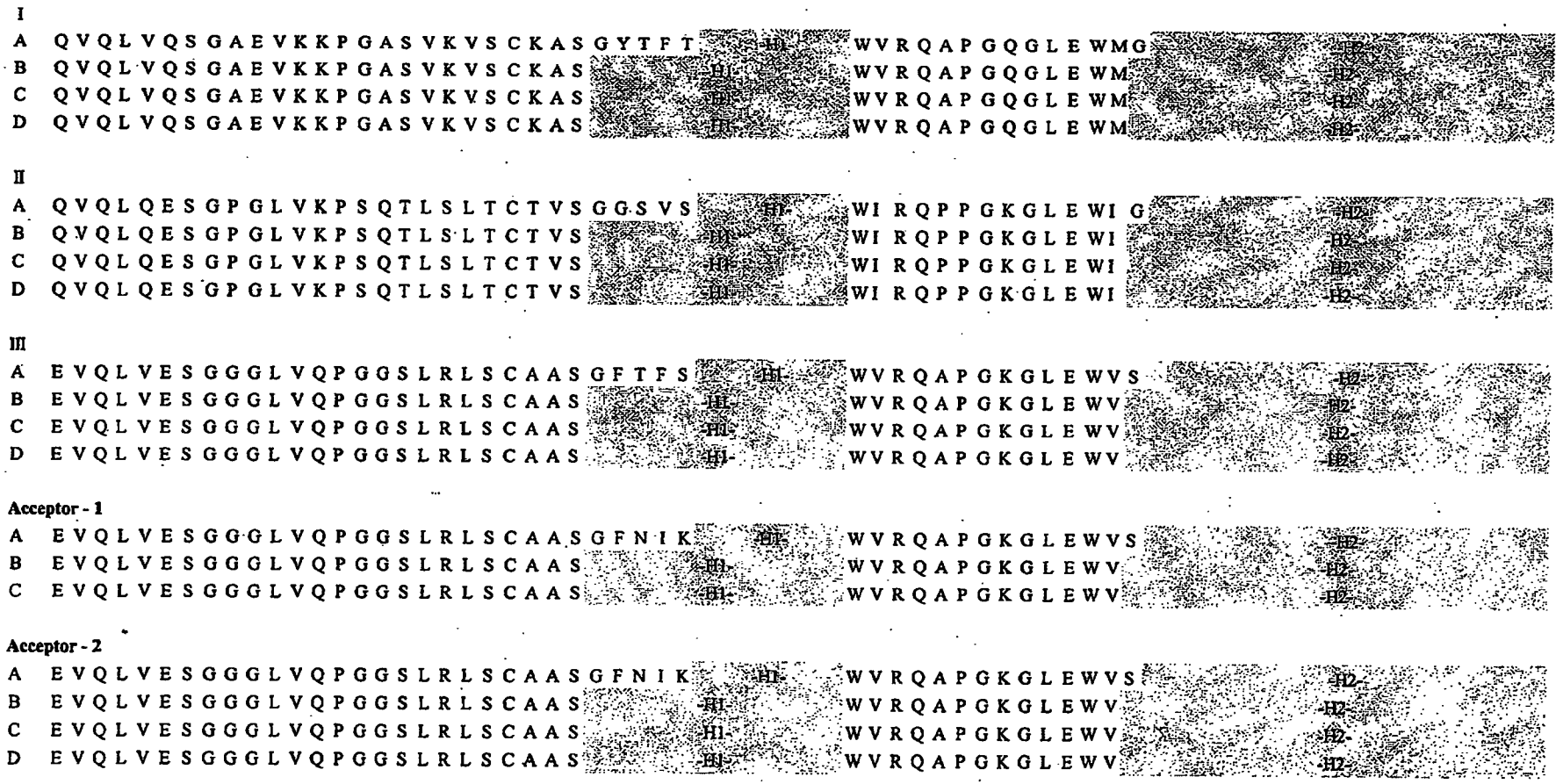


FIG. 3A

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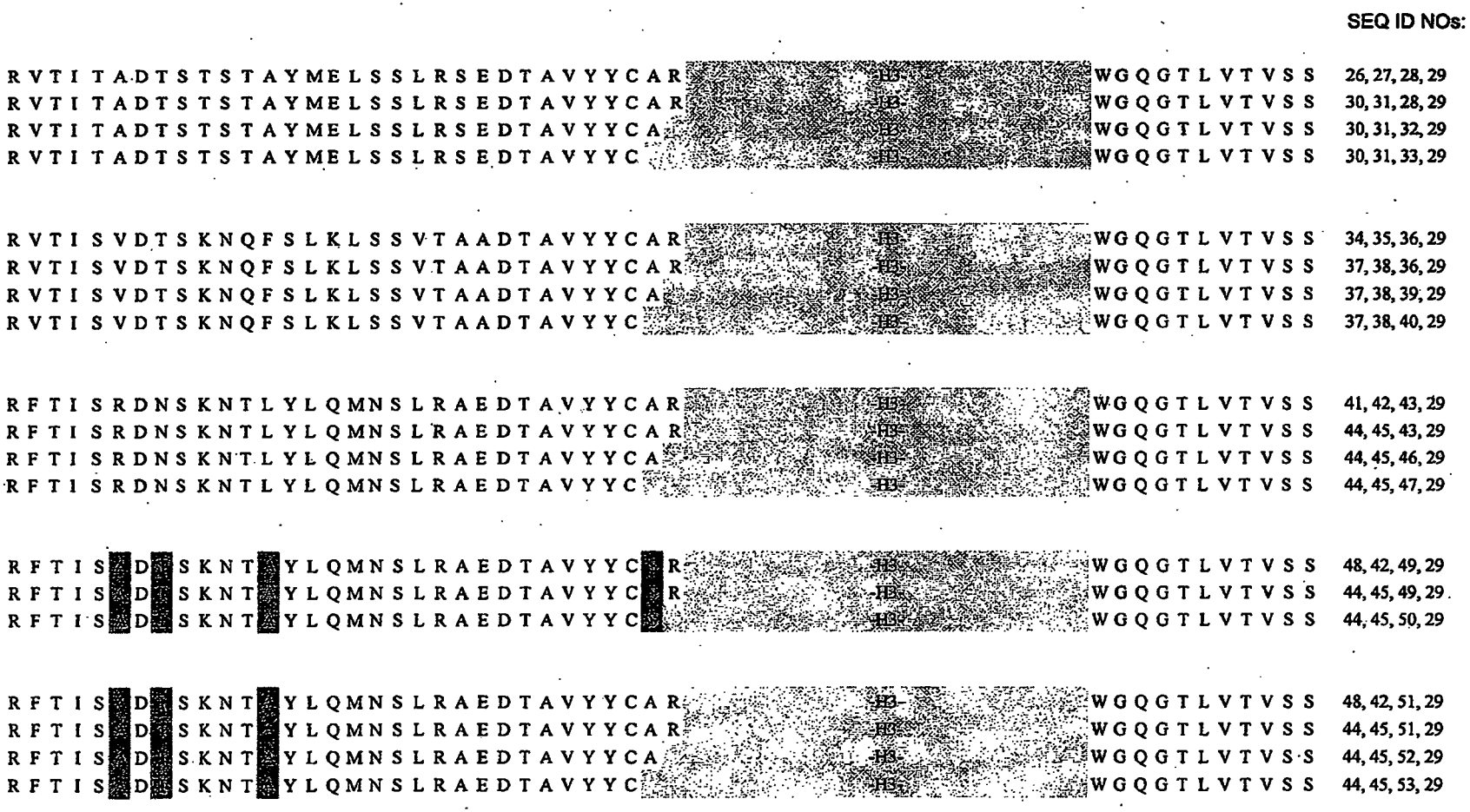


FIG. 3B

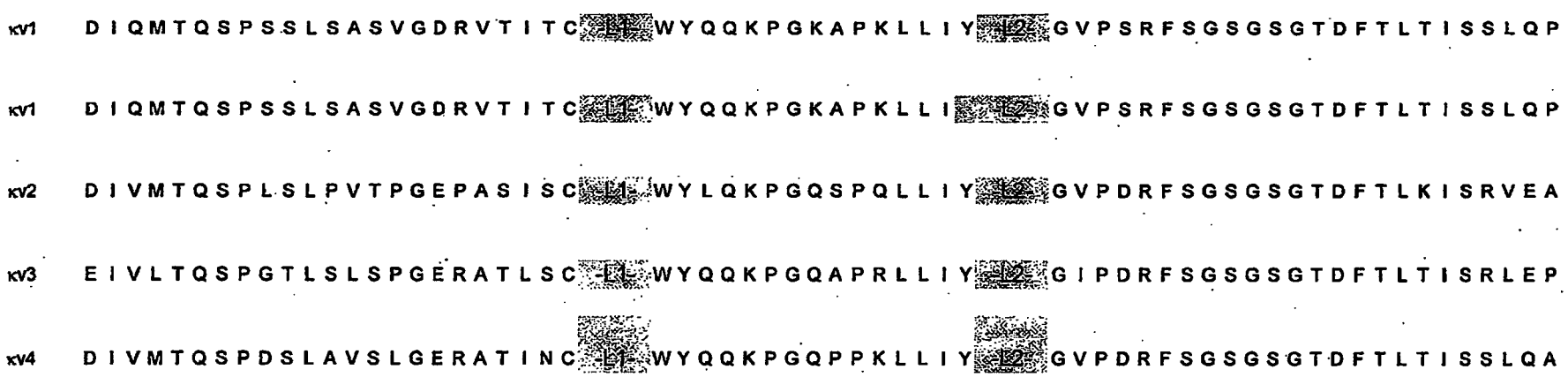


FIG. 4A

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E D F A T Y Y C [redacted] F G Q G T K V E I K	SEQ ID NOs:54, 55, 56, 57
E D F A T Y Y C [redacted] F G Q G T K V E I K	SEQ ID NOs:54, 58, 56, 57
E D V G V Y Y C [redacted] F G Q G T K V E I K	SEQ ID NOs:58, 59, 60, 57
E D F A V Y Y C [redacted] F G Q G T K V E I K	SEQ ID NOs:61, 62, 63, 57
E D V A V Y Y C [redacted] F G Q G T K V E I K	SEQ ID NOs:64, 65, 66, 57

FIG. 4B

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```
          230        240        250        260        270
humIgG1     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
humIgG2     PAP-PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
humIgG3     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV
humIgG4     PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
              ****                                    *     * *

          280        290        300        310        320
humIgG1     DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG2     DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
humIgG3     DGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG4     DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
                           *    *         *                  *

          330        340        350        360        370
humIgG1     APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
                                           D L
humIgG2     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG3     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG4     SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
            **        *                  *

          380        390        400        410        420
humIgG1     EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG2     EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG3     EWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
humIgG4     EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
              *         *    *                  *          * *

          430        440
humIgG1     EALHNHYTQKSLSLSPGK  (SEQ ID NO:85)
humIgG2     EALHNHYTQKSLSLSPGK  (SEQ ID NO:86)
humIgG3     EALHNRFTQKSLSLSPGK  (SEQ ID NO:87)
humIgG4     EALHNHYTQKSLSLSLGK  (SEQ ID NO:88)
              **        *
```

A Kappa light chain constant region consensus sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:89)

Figure 5

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FACS Analysis / Phage Binding Normalized to Display

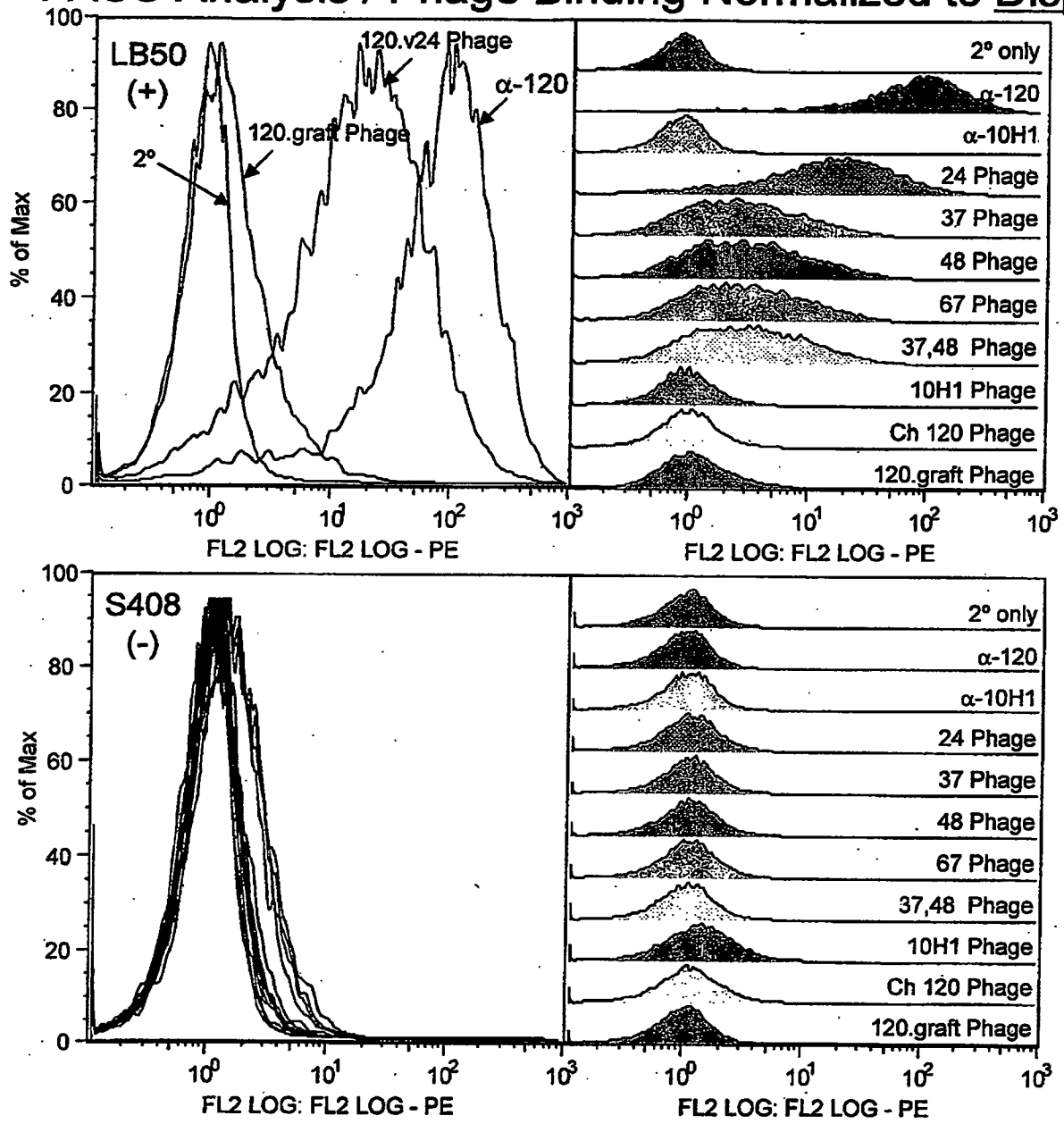


Figure 6

Figure 7A-7C

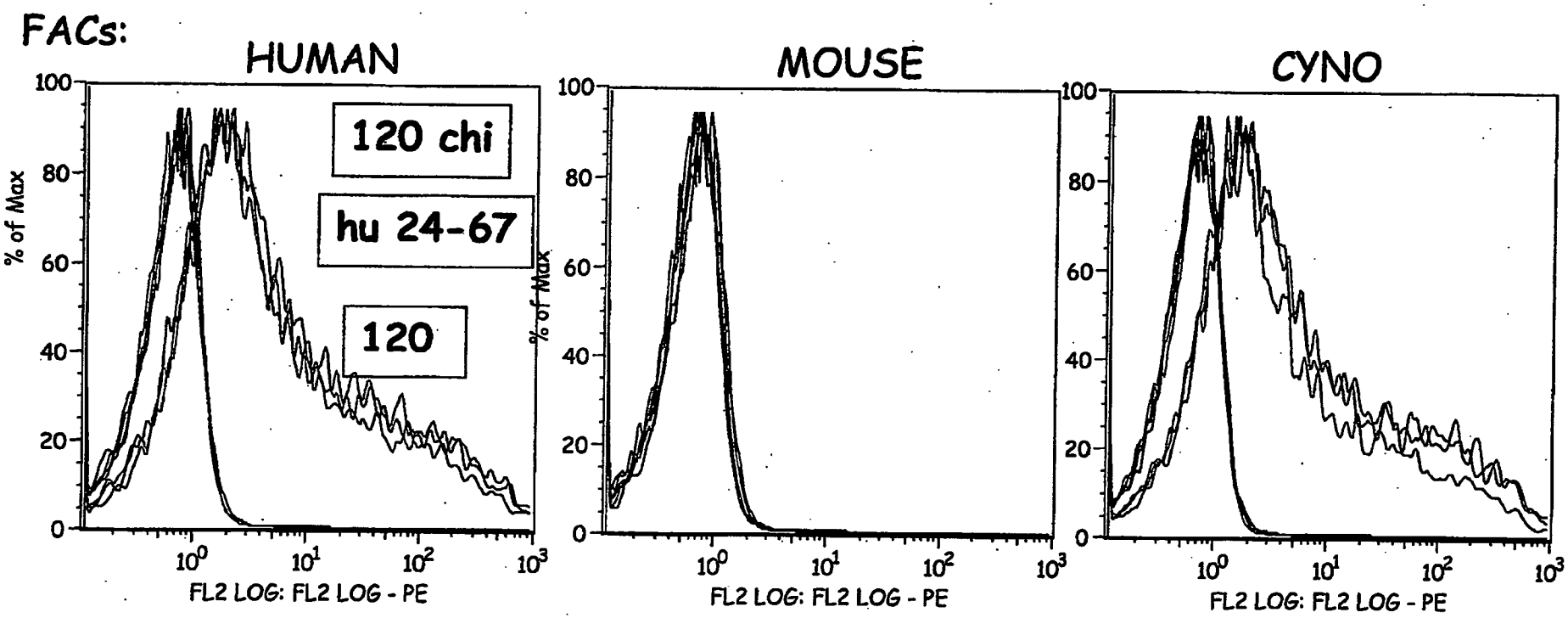


Fig. 7A

Fig. 7B

Fig. 7C

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Figure 7D-7F

FACs:

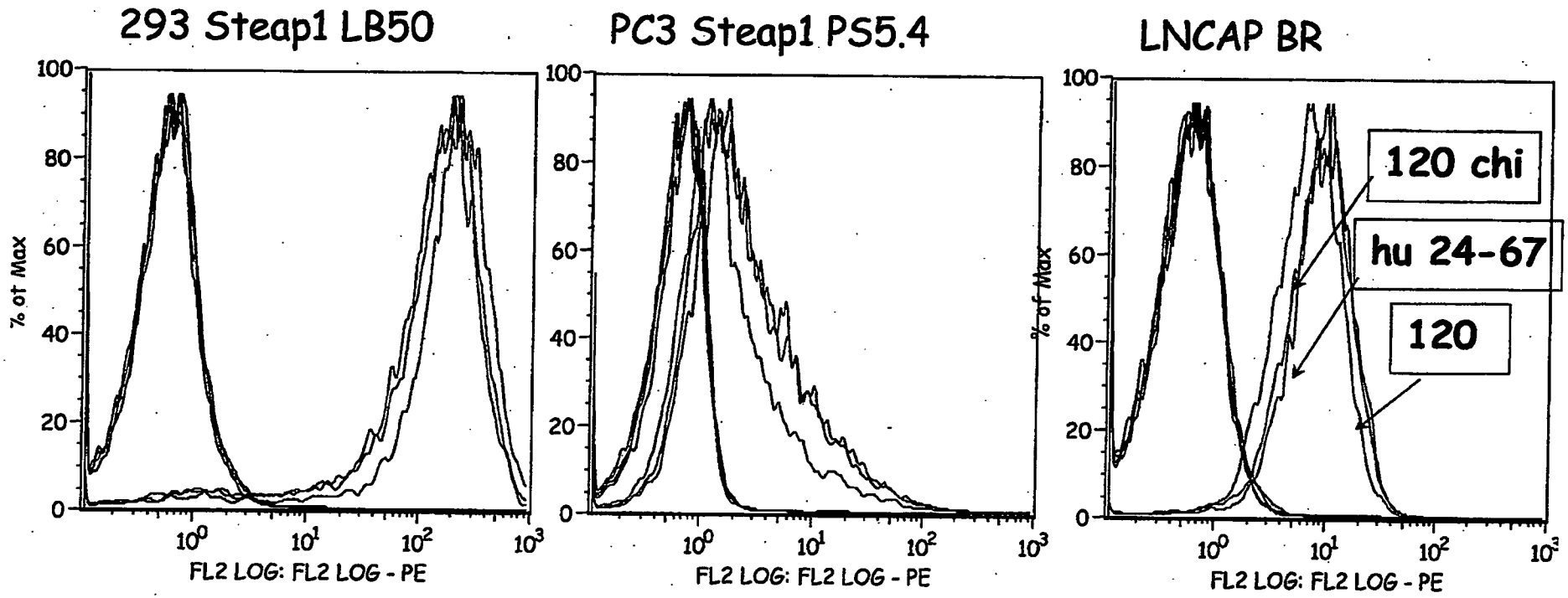


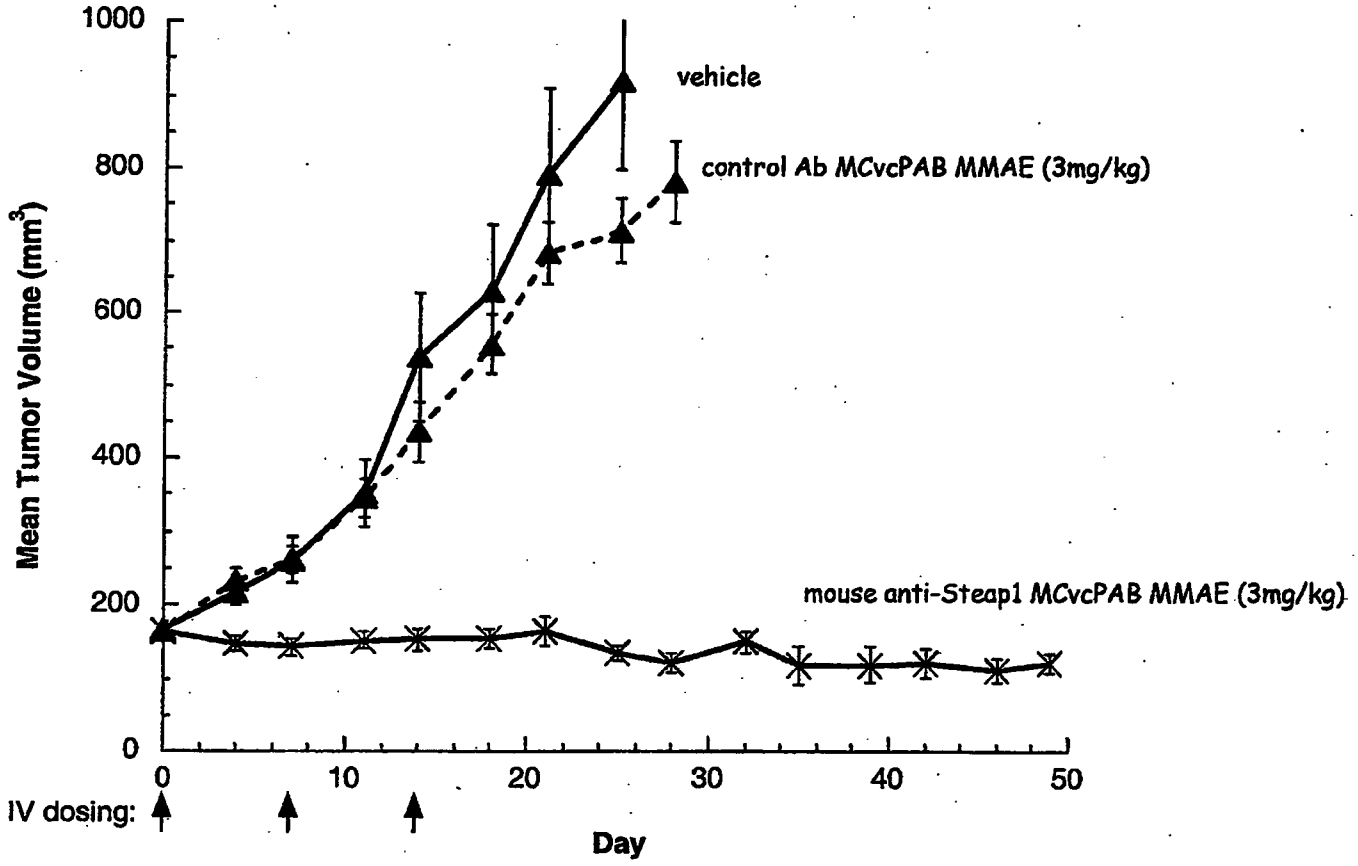
Fig. 7D

Fig. 7E

Fig. 7F

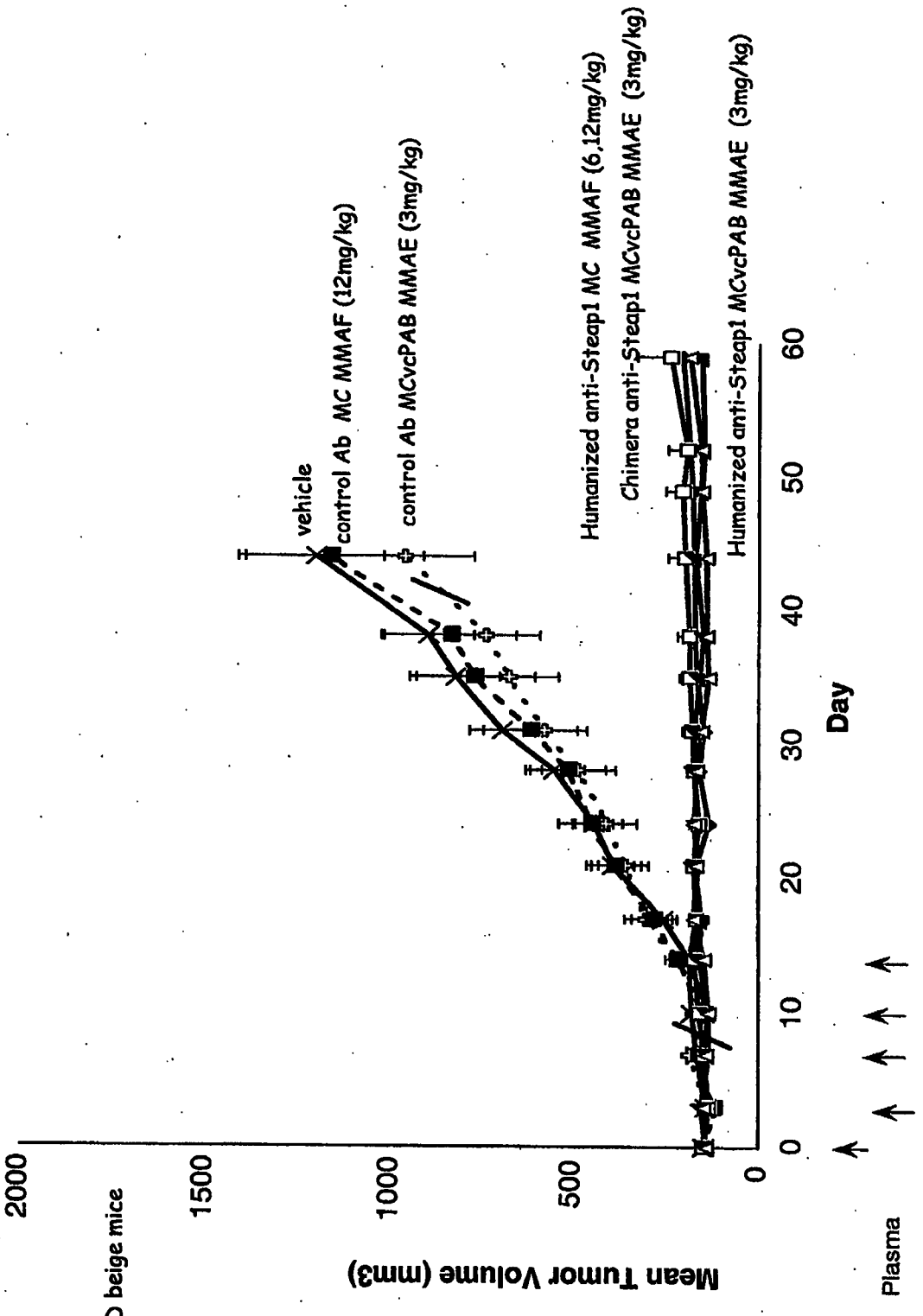
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Figure 8A



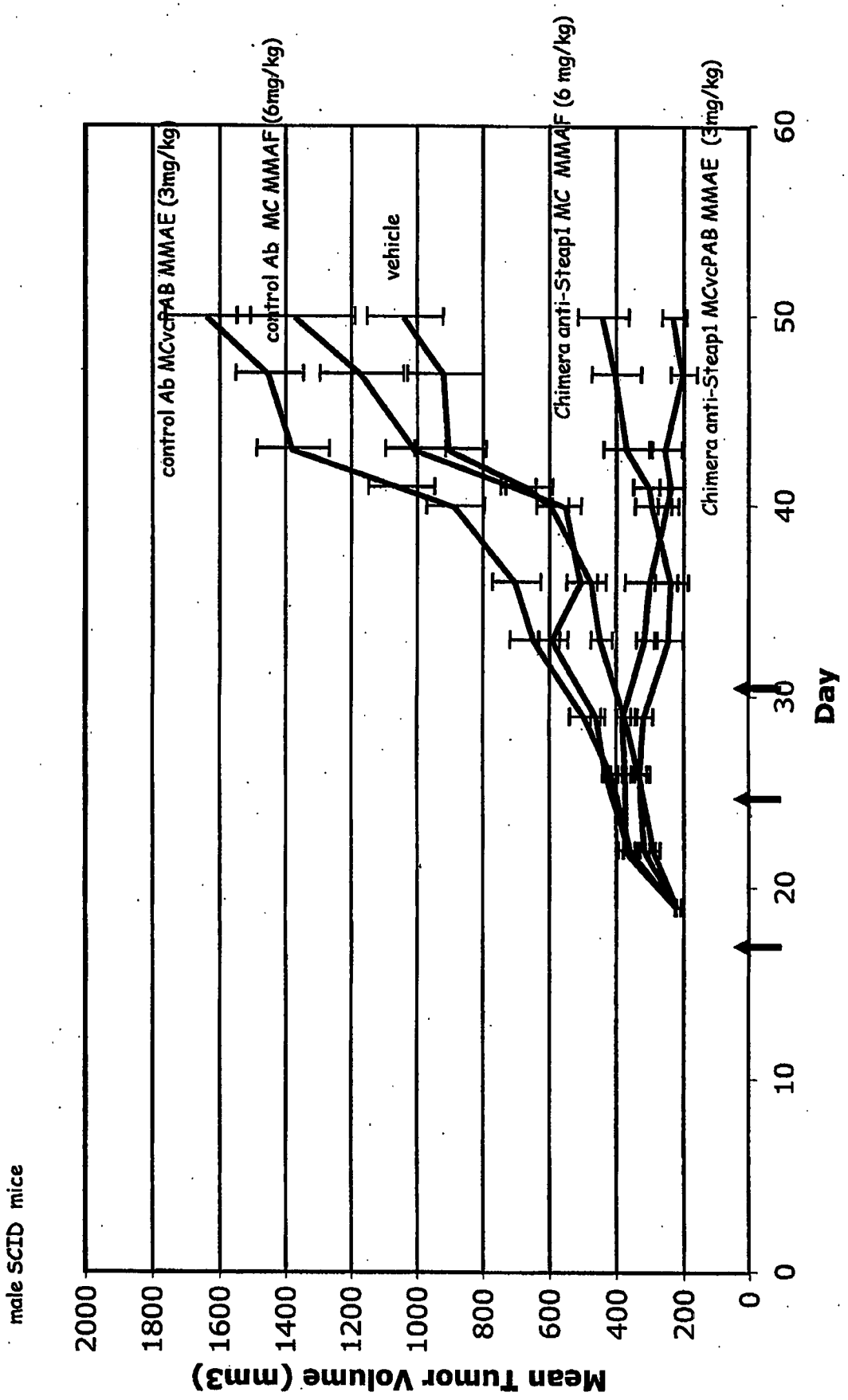
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Figure 8B



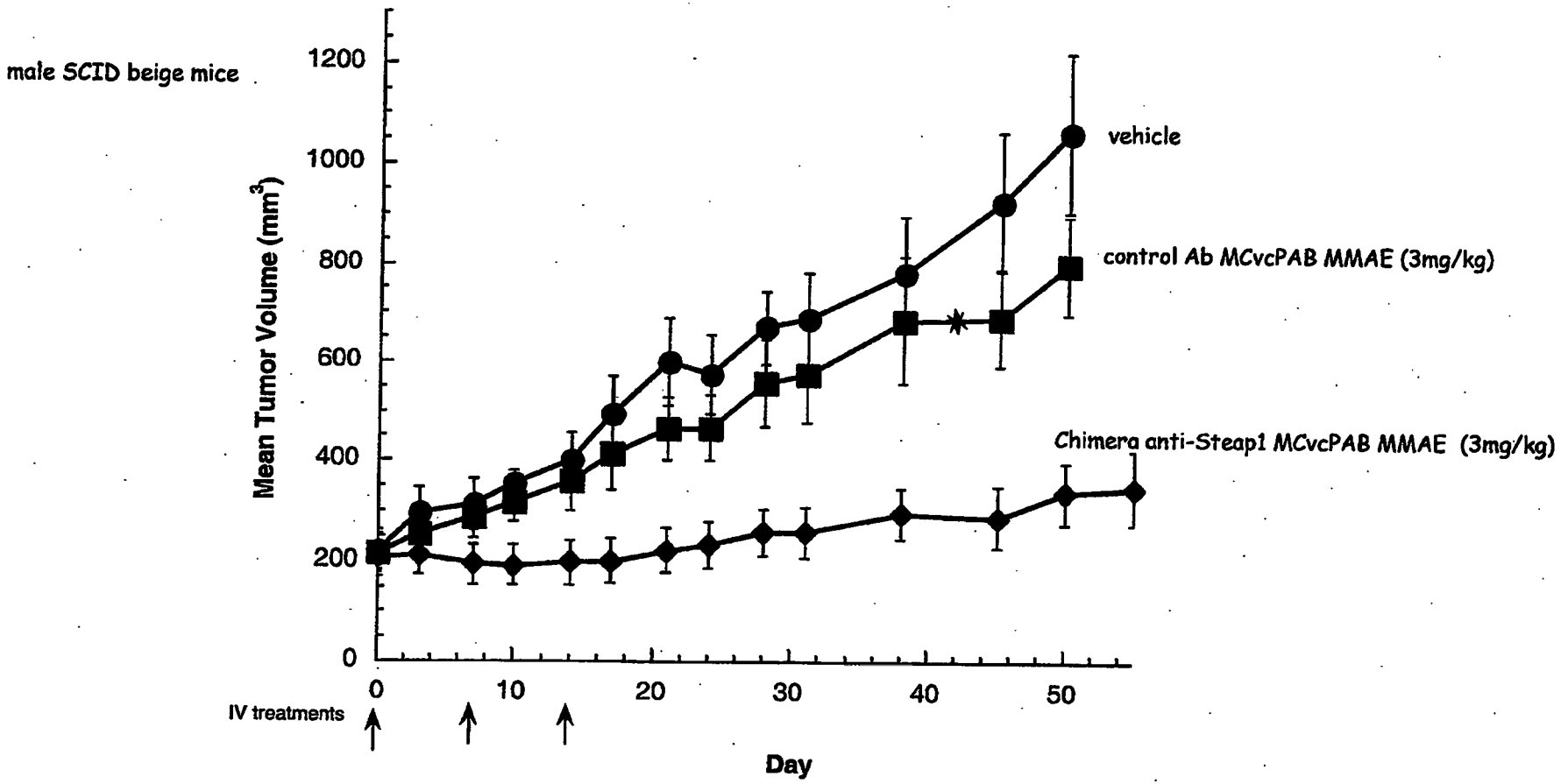
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Figure 9



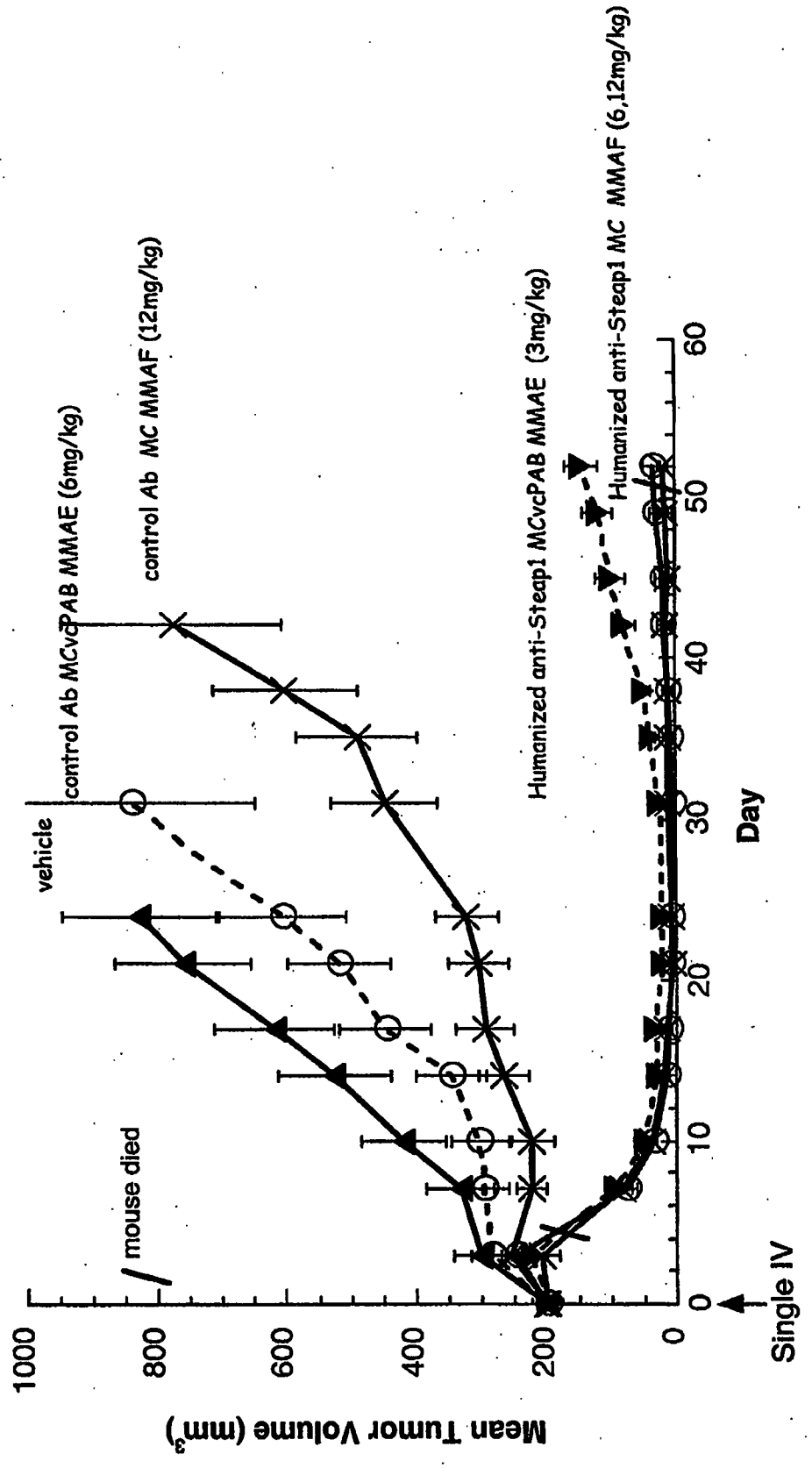
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Figure 10



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Figure 11



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Schematic Diagram of STEAP-1 in the Cell Membrane

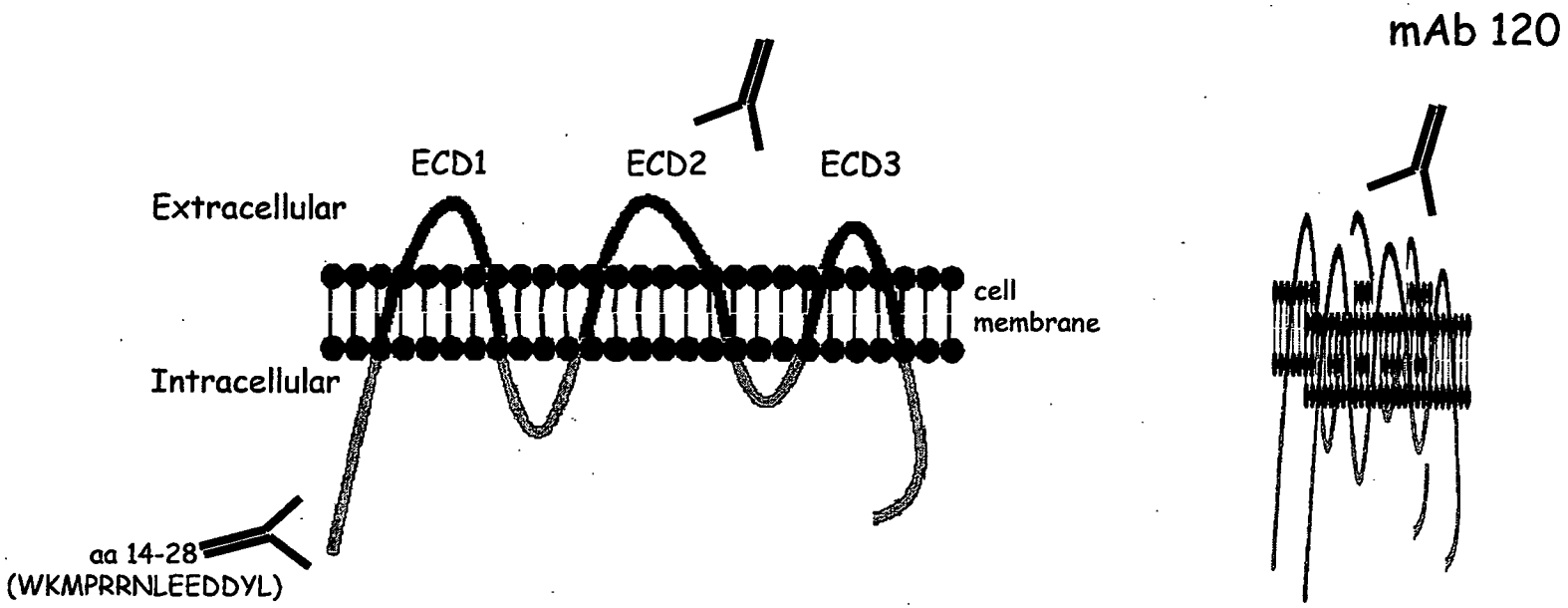


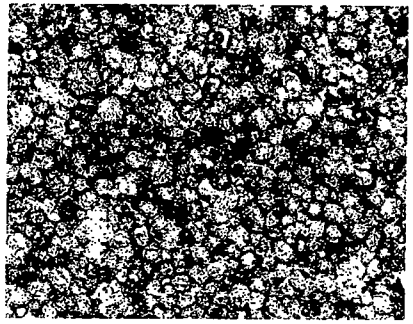
Figure 12

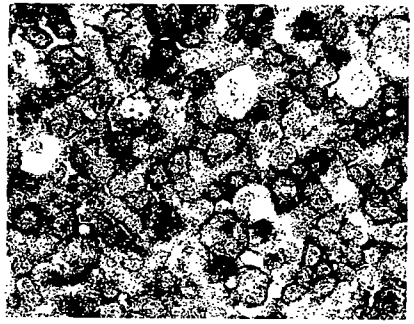
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Expression of STEAP1 in prostate cancer models

Exogenous models *Endogenous models*

293 STEAP1 LB50 PC3 STEAP1 PS5.4 LNCaP LuCAP 77





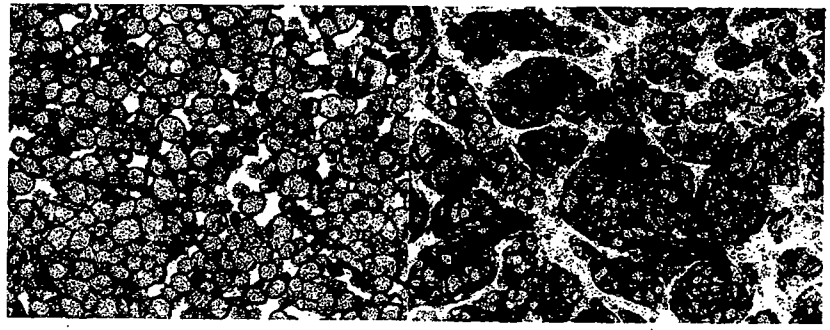


Fig. 13A Fig. 13B Fig. 13C Fig. 13D

Figures 13A-13D

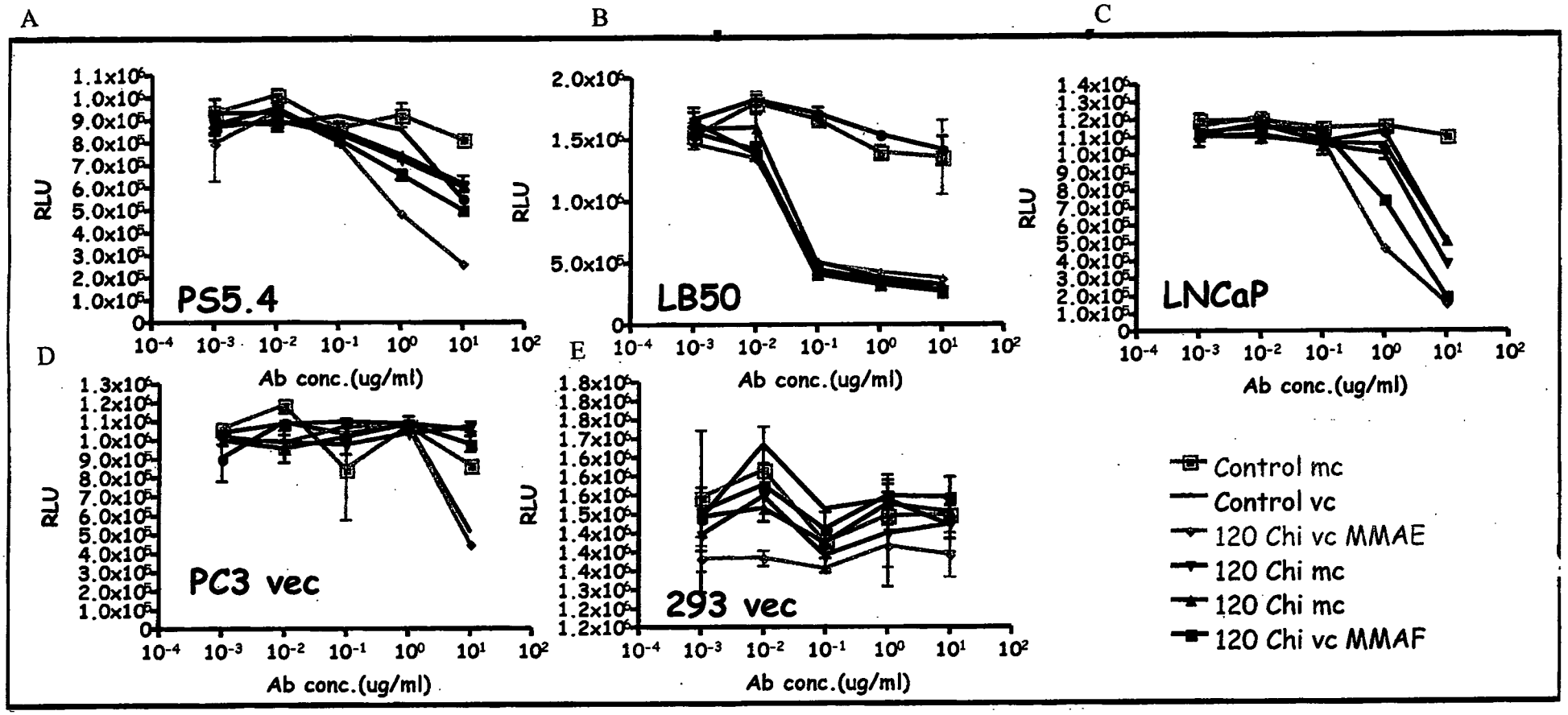


Figure 14

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TRADUCCION OFICIAL No. 2009-05-12-297
DE UN DOCUMENTO ESCRITO EN INGLES

LOS ESTADOS UNIDOS DE AMERICA
A TODOS AQUELLOS QUE VIEREN ESTOS PRESENTES:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas de los Estados Unidos

08 de abril de 2009

POR EL PRESENTE SE CERTIFICA QUE ADJUNTO AL PRESENTE SE ENCUENTRA UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS DE AQUELLOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE CUMPLIO CON LOS REQUISITOS PARA QUE SE LE OTORGUE UNA FECHA DE PRESENTACION BAJO 35 USC 111.

NUMERO DE SOLICITUD: *60/863.295*
FECHA DE PRESENTACION: *27 de octubre de 2006*

EL CODIGO DEL PAIS Y EL NUMERO DE SU SOLICITUD DE PRIORIDAD, PARA SER USADOS PARA PRESENTACION EN EL EXTERIOR BAJO LA CONVENCION DE PARIS, *ES US60/863.295*

Sello del Departamento de Comercio
Oficina de Patentes y Marcas de los Estados Unidos

Por Autoridad del
Subsecretario de Comercio para Propiedad Intelectual y Director de la Oficina de Patentes y Marcas de los Estados Unidos

(firma: ilegible)

E. BORNETT

Funcionario que Certifica

PRESENTADA ELECTRONICAMENTE EL: 27 DE OCTUBRE DE 2006

CARATULA DE SOLICITUD PROVISIONAL

Esta es una solicitud para presentar una SOLICITUD PROVISONAL bajo 37 C.F.R. 1.53(c).

	EXPEDIENTE NUMERO	PR-2412	Marque un signo más (+) dentro de esta casilla →	+

INVENTOR(es)/SOLICITANTE(s)			
APELLIDO	NOMBRE	SEGUNDO NOMBRE	RESIDENCIA (CIUDAD Y ESTADO O PAIS EXTRANJERO)
1) Dennis	Mark		South San Francisco, CA
2) Rubinfeld	Bonee		South San Francisco, CA
3) Polakis	Paul		South San Francisco, CA
4) Jakobovits	Aya		Santa Monica, CA

IDENTIFICADOR DE LA SOLICITUD (si no se nombra ningún INVENTOR(s)/SOLICITANTE(s)
TITULO DE LA INVENCION *(280 caracteres max)*
ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS
DIRECCION DE CORRESPONDENCIA

CLIENTE NO. 09157			Número de Teléfono (650) 225-2066		
			Número de Fax (650) 952-9881		
ESTADO	California	CODIGO POSTAL	94080-4990	PAIS	EU

PARTES DE LA SOLICITUD ADJUNTAS Y CALCULO DE DERECHOS

Número Presentado		Número Extra		Tasa	Derechos Básicos 37 CFR 1.16(a)
					$200
Total Páginas (Incluyendo Figuras y Listado de Secuencia impreso, ya sea registrado con o sin un diskette/CD)	177	- 100 =	77	$250	$500.00
DERECHO DE PRESENTACION PROVISIONAL TOTAL					$700.00

METODO DE PAGO (*marque uno)*

☐ Se adjunta un cheque o giro postal para cubrir los derechos de presentación Provisional.

☒ Por el presente el Comisionado está autorizado para cargar derechos de presentación y acreditar Cuenta de Depósito No. 07-0630. Por el presente el Comisionado está autorizado para cargar cualquier derecho adicional requerido bajo 37 CFR §1.16 y 1.17, o acreditar sobrepago a la Cuenta de Depósito No. 07-0630. Se adjunta un duplicado de esta hoja.

La invención fue hecha por una agencia del gobierno de los Estados Unidos o bajo un contrato con una agencia del gobierno de los Estados Unidos.

☒ No.

☐ Sí, el nombre de la agencia del gobierno de los EU y el número(s) del contrato del gobierno es(son):

Presentado respetuosamente,

GENENTECH, INC.

Fecha: 27 de octubre de 2006

Por: (firma: ilegible)

Deirdre L. Conley, Ph.D.

Reg. No. 36.487

Teléfono No. (650) 225-2066

Recibo de Reconocimiento Electrónico	
ID EFS:	1279601
Número de Solicitud:	60863295
Número de Solicitud Internacional:	
Número de Confirmación:	4499
Título de la Invención:	ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS
Primer Inventor Nombrado/Nombre del Solicitante:	Mark Dennis
Número del Cliente:	09157
Registrador:	Deirdre L. Conley
Registrador Autorizado por:	
Número de Expediente del Apoderado:	PR2412
Fecha de Recepción:	27-OCT-2006
Fecha de Presentación:	
Estampilla de Tiempo:	18:16:14
Tipo de Solicitud:	Provisional
Información de Pago:	
Presentada con Pago	no
Listas de Archivos:	

Número del Documento	Descripción del Documento	Nombre del Archivo	Tamaño del Archivo (Bytes)	Multi Partes/.zip	Páginas (si aplica)

1	Carta de transmisión	PR2412_SOLICITUD_PROVISIONAL_CARATULA.pdf	48095	no	1
2		PR2412_US_SOLICITUD.pdf	9056977	sí	158

Descripción Multipartes/archivo PDF en descripción .zip

Descripción del Documento	Inicio	Fin
Especificación	1	150
Reivindicaciones	152	157
Resumen	158	158

Advertencias:

Información:

3	Dibujos	PR2412_DIBUJOS.pdf	662488	no	16

Advertencias:

Información:

Tamaño Total Archivos (en bytes):	9767560

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Nuevas Solicitudes Bajo 35 U.S.C. 111

Si se presenta una nueva solicitud y la solicitud incluye los componentes necesarios para una fecha de presentación (ver 37 CFR 1.53(b)-(d) y MPEP 506), un Recibo de Presentación (37 CFR 1.54) será expedido en el curso debido y la fecha mostrada en este Recibo de Reconocimiento establecerá la fecha de presentación de la solicitud.

Fase Nacional de una Solicitud Internacional bajo 35 U.S.C. 371

Si una presentación a tiempo para registrar una solicitud internacional en la fase nacional cumple con las condiciones de 35 U.S.C. 371 y otros requerimientos que aplican se expedirá un Formato PCT/DO/EO/903 que indica aceptación de la solicitud como una presentación de la fase nacional bajo 35 U.S.C. 371 además del Recibo de Presentación, en su curso debido.

Traducido según mi mejor saber y entender, este día 12 de mayo de 2009, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTORA OFICIAL
RES. 2177/89 MINJUSTICIA

ANITA ESCOBAR DE TAMAYO

PA 7178481

THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 08, 2009

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/863,295***
FILING DATE: ***October 27, 2006***

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS ***US60/863,295***

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

E. BORNETT
Certifying Officer

FILED ELECTRONICALLY ON: 27 October 2006

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

DOCKET NUMBER	PR2412	Type a plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
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APPLICATION IDENTIFIER (if no INVENTOR(s)/APPLICANT(s) are named)

TITLE OF THE INVENTION *(280 characters max)*

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

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METHOD OF PAYMENT *(check one)*

☐ A check or money order is enclosed to cover the Provisional filing fees.

☒ The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 07-0630. The Commissioner is hereby authorized to charge any additional fees required under 37 CFR §1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. A duplicate of this sheet is enclosed.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number(s) is(are):

Respectfully submitted,
GENENTECH, INC.

Date: October 27, 2006

By: _____

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Electronic Acknowledgement Receipt

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Document Description	**Start**	**End**
Specification	1	150
Claims	151	157
Abstract	158	158

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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.

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ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates to anti-STEAP-1 antibodies and immunconjugates thereof. The invention further relates to methods of using anti-STEAP-1 antibodies and immunconjugates thereof.

BACKGROUND

FIELD OF THE INVENTION

The present invention relates to anti-STEAP-1 antibody compositions and methods of killing STEAP-1 expressing cancers cells.

BACKGROUND OF THE INVENTION

In humans, prostate cancer is one of the most commonly diagnosed malignancies in males and is the second leading cause of cancer related death in men. The American Cancer Society estimates that for the year 2000, 180,400 new cases of prostate cancer will be diagnosed with 31,900 deaths from the disease. In advanced stages, prostate cancer metastasizes to the bone. While advances in early diagnosis and treatment of locally confined tumors have been achieved, prostate cancer is incurable once it has metastasized. Patients with metastatic prostate cancer on hormonal therapy will eventually develop an androgen-refractory (androgen independent) state that will lead to disease progression and death. Currently, prostate-specific antigen (PSA) is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer. However, widespread use of PSA as a tool for screening is controversial since PSA fails to discriminate accurately between benign and malignant prostate disease.

Depending on the stage of the cancer, prostate and bladder cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, chemotherapy, androgen deprivation (e.g., hormonal therapy) in the case of prostate cancer. While surgical or radiation therapy significantly improves survival in patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences following hormone ablation. The majority of patients who undergo hormone therapy progress to develop androgen-independent disease. Currently, there is no effective treatment for the 20-40% of prostate cancer patients who develop recurrent disease after surgery or radiation therapy, or for those in whom the cancer has

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metastasized at the time of diagnosis. Chemotherapy has its toxic side effects, especially in elderly patients. Development of new forms of therapy especially for disease refractory to androgen deprivation is an urgent need in the management of prostatic carcinoma.

The identification of a novel cell surface antigen, STEAP-1 has been described (see US6,329,503). STEAP-1 is member of cell surface serpentine transmembrane antigens. It is expressed predominantly in the prostate cancer, and thus members of this family have been tered "STEAP" (Six Transmembrane Epithelial Antigens of the Prostate). Human STEAP proteins exhibit a high degree of structural conservation within the family by show no significant structural homology to any known human proteins. STEAP-1 appears to be a type IIIa membrane protein expressed predominantly in prostate cells in normal human tissues. Structurally, STEAP-1 is a 339 amino acid protein characterized by a molecular topology of six transmembrane domains and intracellular N- and C-termini, suggesting that it folds in a "serpentine" manner into three extracellular and two intracellular loops. STEAP-1 protein expression is maintained at high levels across various states of prostate cancer. STEAP-1 is highly over-expressed in other human cancers such as lung and colon. Murine antibodies have been raised to human STEAP-1 fragments and the antibodies were shown to bind STEAP-1 on the cell surface (see US20040253232A1).

Antibody-based therapy has proved very effective in the treatment of various cancers. For example, HERCEPTIN® and RITUXAN® (both from Genentech, S. San Francisco), have been used successfully to treat breast cancer and non-Hodgkin's lymphoma, respectively. HERCEPTIN® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) proto-oncogene. HER2 protein overexpression is observed in 25-30% of primary breast cancers. RITUXAN® is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. Both these antibodies are produced in CHO cells.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4975278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin,

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methotrexate, and vindesine (Rowland et al., Cancer Immunol. Immunother. 21:183-87 (1986)). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Kerr et al (1997) Bioconjugate Chem. 8(6):781-784; Mandler et al (2000) Journal of the 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). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition (Meyer, D.L. and Senter, P.D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" in Annual Reports in Medicinal Chemistry, Vol 38 (2003) Chapter 23, 229-237). Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is being developed for the treatment of cancers that express CanAg antigen, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The same maytansinoid drug moiety, DM1, was linked through a non-disulfide linker, SMCC, to a mouse murine monoclonal antibody, TA.1 (Chari et al. (1992) Cancer Research 52:127-131). This conjugate was reported to be 200-fold less potent than the corresponding disulfide linker conjugate. The SMCC linker was considered therein to be "noncleavable."

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Several short peptidic compounds have been isolated from the marine mollusk, Dolabella auricularia, and found to have biological activity (Pettit et al (1993) Tetrahedron 49:9151; Nakamura et al (1995) Tetrahedron Letters 36:5059-5062; Sone et al (1995) Journal Org Chem. 60:4474). Analogs of these compounds have also been prepared, and some were found to have biological activity (for a review, see Pettit et al (1998) Anti-Cancer Drug Design 13:243-277). For example, auristatin E (US 5635483) is a synthetic analogue of the marine natural product Dolastatin 10, an agent that inhibits tubulin polymerization by binding to the same site on tubulin as the anticancer drug vincristine (G. R. Pettit, (1997) Prog. Chem. Org. Nat. Prod. 70:1-79). Dolastatin 10, auristatin PE, and auristatin E are linear peptides having four amino acids, three of which are unique to the dolastatin class of compounds, and a C-terminal amide.

The auristatin peptides, auristain E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to: (i) chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas); (ii) cAC10 which is specific to CD30 on hematological malignancies (Klussman, et al (2004), Bioconjugate Chemistry 15(4):765-773; Doronina et al (2003) Nature Biotechnology 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco et al (2003) Blood 102(4):1458-1465; US 2004/0018194; (iii) anti-CD20 antibodies such as Rituxan® (rituximab) (WO 04/032828) for the treatment of CD20-expressing cancers and immune disorders; (iv) anti-EphB2 antibodies 2H9 and anti-IL-8 for treatment of colorectal cancer (Mao, et al (2004) Cancer Research 64(3):781-788); (v) E-selectin antibody (Bhaskar et al (2003) Cancer Res. 63:6387-6394); and (vi) other anti-CD30 antibodies (WO 03/043583). Monomethylauristatin (MMAE) has also been conjugated to 2H9, an antibody against EphB2R which is a type 1 TM tyrosine kinase receptor with close homology between mouse and human, and is over-expressed in colorectal cancer cells (Mao et al (2004) Cancer Res. 64:781-788).

Monomethylauristatin MMAF, a variant of auristatin E (MMAE) with a phenylalanine at the C-terminus (US 5767237; US 6124431), has been reported to be less potent than MMAE, but more potent when conjugated to monoclonal antibodies (Senter et al, Proceedings fo the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004). Auristatin F phenylene diamine (AFP); a phenylalanine variant of MMAE was linked to an anti-CD70 mAb, 1F6, through the C-terminus of 1F6 via a phenylene diamine spacer (Law et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 625, presented March 28, 2004).

There exists a need in the art for additional drugs to treat various cancers such as cancers and metatases of cancers in the prostate, lung and colon. Particularly useful drugs for this purpose include

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prostate, lung or colon cell targeted anti-STEAP-1 antibody-drug conjugates having a significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

The recitation of any reference in this application is not an admission that the reference is prior art to this application. All references cited herein, including patents, patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

The invention provides anti-STEAP-1 antibodies and methods of using the same.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) or a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9). In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) and a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9).

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79.

In one aspect, the antibody comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of

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SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79. In an embodiment, the antibody further comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In certain embodiments, a polynucleotide encoding any of the above antibodies is provided. In one embodiment, a vector comprising the polynucleotide is provided. In one embodiment, a host cell comprising the vector is provided. In one embodiment, the host cell is eukaryotic. In one embodiment, the host cell is a Chinese hamster ovary (CHO) cell. In one embodiment, a method of making an anti-STEAP-1 antibody is provided, wherein the method comprises culturing the host cell under conditions suitable for expression of the polynucleotide encoding the antibody, and isolating the antibody.

In one aspect, an antibody that binds to STEAP-1 expressed on the surface of a cell is provided. In one embodiment, the antibody binds to an epitope within a region of human or mouse STEAP-1. In one embodiment, the cell is mammalian cell. In one embodiment, the cell is a human cell. In one embodiment, the cell is a cancer cell. In one embodiment the cell is a prostate, lung or colon cell. In one embodiment the cancer cell is a prostate cancer cell. In another embodiment, the cell cell from a metastisis of a primary prostate, lung or colon cancer.

In certain embodiments, any of the above antibodies is a monoclonal antibody. In one embodiment, the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one embodiment, the antibody is humanized. In one embodiment, the antibody is human.

In one aspect, a method of detecting the presence of STEAP-1 in a biological sample is provided, the method comprising contacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1. In one embodiment, the biological sample comprises prostate cells. In one embodiment, the biological sample is from a mammal experiencing or suspected of experiencing a prostate cell disorder and/or a cell proliferative disorder of cells or tissues including, but not limited to, prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma as well as metastases of primary prostate, lung, colon, bladder, and ovarian cancers and Ewing's sarcoma. See, for example, (see US6,329,503; and Rodeberg, D.A. et al., Clin. Cancer Res. 11(12):4545-4552 (2005)).

In one aspect, a method of diagnosing a cell proliferative disorder associated with increased expression of STEAP-1 is provided, the method comprising contacting a test cell with any of the above

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antibodies; determining the level of expression of STEAP-1 by detecting binding of the antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell, wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a cell proliferative disorder associated with increased expression of STEAP-1. In one embodiment, the test cell is a cell from a patient suspected of having a cell proliferative disorder, such as a prostate proliferative disorder. In one embodiment, the cell proliferative disorder is selected from prostate cell disorders including but not limited to prostate cancer. In one embodiment, the method comprises determining the level of expression of STEAP-1 on the surface of the test cell (such as, for example, a prostate cancer cell) and comparing the level of expression of STEAP-1 on the surface of the test cell with the level of expression of STEAP-1 on the surface of the control cell (such, for example, as a normal prostate cell other than an abnormally proliferating prostate cell).

In one aspect, a method of diagnosing a cell proliferative disorder associated with an increase in cells, such as prostate cells, expressing STEAP-1 is provided, the method comprising contacting a test cells in a biological sample with any of the above antibodies; determining the level of antibody bound to test cells in the sample by detecting binding of the antibody to STEAP-1; and comparing the level of antibody bound to cells in a control sample, wherein the level of antibody bound is normalized to the number of STEAP-1-expressing cells in the test and control samples, and wherein a higher level of antibody bound in the test sample as compared to the control sample indicates the presence of a cell proliferative disorder associated with cells expressing STEAP-1.

In one aspect, a method of detecting soluble STEAP-1 in blood or serum, the method comprising contacting a test sample of blood or serum from a mammal suspected of experiencing a prostate cell proliferative disorder with an anti-STEAP-1 antibody of the invention and detecting a increase in soluble STEAP-1 in the test sample relative to a control sample of blood or serum from a normal mammal. In an embodiment, the method of detecting is useful as a method of diagnosing a prostate cell proliferative disorder associated with an increase in soluble STEAP-1 in blood or serum of a mammal.

In one aspect, the antibodies of the invention include cysteine engineered antibodies where one or more amino acids of a parent antibody are replaced with a free cysteine amino acid as disclosed in WO2006/034488 (herein incorporated by reference in its entirety). A cysteine engineered antibody comprises one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0. A free cysteine amino acid is a cysteine residue which has been engineered into the parent antibody and is not part of a disulfide bridge. Cysteine engineered antibodies are useful for attachment of cytotoxic and/or imaging compounds at the site of the engineered cysteine through, for example, a maleimide or haloacetyl.

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The nucleophilic reactivity of the thiol functionality of a Cys residue to a maleimide group is about 1000 times higher compared to any other amino acid functionality in a protein, such as amino group of lysine residues or the N-terminal amino group. Thiol specific functionality in iodoacetyl and maleimide reagents may react with amine groups, but higher pH (>9.0) and longer reaction times are required (Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London).

Cysteine engineered antibodies may be useful in the treatment of cancer and include antibodies specific for cell surface and transmembrane receptors, and tumor-associated antigens (TAA). Such antibodies may be used as naked antibodies (unconjugated to a drug or label moiety) or as antibody-drug conjugates (ADC). Cysteine engineered antibodies of the invention may be site-specifically and efficiently coupled with a thiol-reactive reagent. The thiol-reactive reagent may be a multifunctional linker reagent, a capture label reagent, a fluorophore reagent, or a drug-linker intermediate. The cysteine engineered antibody may be labeled with a detectable label, immobilized on a solid phase support and/or conjugated with a drug moiety. Thiol reactivity may be generalized to any antibody where substitution of amino acids with reactive cysteine amino acids may be made within the ranges in the light chain selected from amino acid ranges: L-10 to L-20; L-38 to L-48; L-105 to L-115; L-139 to L-149; L-163 to L-173; and within the ranges in the heavy chain selected from amino acid ranges: H-35 to H-45; H-83 to H-93; H-114 to H-127; and H-170 to H-184, and in the Fc region within the ranges selected from H-268 to H-291; H-319 to H-344; H-370 to H-380; and H-395 to H-405, where the numbering of amino acid positions begins at position 1 of the Kabat numbering system (Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and continues sequentially thereafter as disclosed in WO2006034488. Thiol reactivity may also be generalized to certain domains of an antibody, such as the light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. Cysteine replacements resulting in thiol reactivity values of 0.6 and higher may be made in the heavy chain constant domains α, δ, ε, γ, and μ of intact antibodies: IgA, IgD, IgE, IgG, and IgM, respectively, including the IgG subclasses: IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. Such antibodies and their uses are disclosed in WO2006/034488.

Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, transmembrane proteins, signalling proteins, cell survival regulatory factors, cell proliferation regulatory factors, molecules associated with (for e.g., known or suspected to contribute functionally to) tissue development or differentiation,

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lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in vasculogenesis and molecules associated with (for e.g., known or suspected to contribute functionally to) angiogenesis.

An antibody of the invention may be conjugated to other thiol-reactive agents in which the reactive group is, for example, a maleimide, an iodoacetamide, a pyridyl disulfide, or other thiol-reactive conjugation partner (Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Hermanson, G. in Bioconjugate Techniques (1996) Academic Press, San Diego, pp. 40-55, 643-671). The partner may be a cytotoxic agent (e.g. a toxin such as doxorubicin or pertussis toxin), a fluorophore such as a fluorescent dye like fluorescein or rhodamine, a chelating agent for an imaging or radiotherapeutic metal, a peptidyl or non-peptidyl label or detection tag, or a clearance-modifying agent such as various isomers of polyethylene glycol, a peptide that binds to a third component, or another carbohydrate or lipophilic agent.

In one aspect, antibodies of the invention may be conjugated with any label moiety which can be covalently attached to the antibody through a reactive moiety, an activated moiety, or a reactive cysteine thiol group (Singh et al (2002) Anal. Biochem. 304:147-15; Harlow E. and Lane, D. (1999) Using Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press, Cold Spring Harbor, NY; Lundblad R.L. (1991) Chemical Reagents for Protein Modification, 2nd ed. CRC Press, Boca Raton, FL). The attached label may function to: (i) provide a detectable signal; (ii) interact with a second label to modify the detectable signal provided by the first or second label, e.g. to give FRET (fluorescence resonance energy transfer); (iii) stabilize interactions or increase affinity of binding, with antigen or ligand; (iv) affect mobility, e.g. electrophoretic mobility or cell-permeability, by charge, hydrophobicity, shape, or other physical parameters, or (v) provide a capture moiety, to modulate ligand affinity, antibody/antigen binding, or ionic complexation.

Labelled cysteine engineered antibodies may be useful in diagnostic assays, e.g., for detecting expression of an antigen of interest in specific cells, tissues, or serum. For diagnostic applications, the antibody will typically be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

Radioisotopes (radionuclides), such as ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Radioisotope labelled antibodies are useful in receptor targeted imaging experiments. The antibody can be labeled with ligand reagents that bind, chelate or otherwise complex a radioisotope metal where the reagent is reactive with the engineered cysteine thiol of the

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antibody, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, NY, Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, TX). Radionuclides can be targetted via complexation with the antibody-drug conjugates of the invention (Wu et al (2005) Nature Biotechnology 23(9):1137-1146).

Linker reagents such as DOTA-maleimide (4-maleimidobutyramidobenzyl-DOTA) can be prepared by the reaction of aminobenzyl-DOTA with 4-maleimidobutyric acid (Fluka) activated with isopropylchloroformate (Aldrich), following the procedure of Axworthy et al (2000) Proc. Natl. Acad. Sci. USA 97(4):1802-1807). DOTA-maleimide reagents react with the free cysteine amino acids of the cysteine engineered antibodies and provide a metal complexing ligand on the antibody (Lewis et al (1998) Bioconj. Chem. 9:72-86). Chelating linker labelling reagents such as DOTA-NHS (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono (N-hydroxysuccinimide ester) are commercially available (Macrocyclics, Dallas, TX). Receptor target imaging with radionuclide labelled antibodies can provide a marker of pathway activation by detection and quantitation of progressive accumulation of antibodies in tumor tissue (Albert et al (1998) Bioorg. Med. Chem. Lett. 8:1207-1210). The conjugated radio-metals may remain intracellular following lysosomal degradation.

Metal-chelate complexes suitable as antibody labels for imaging experiments are disclosed: US 5342606; US 5428155; US 5316757; US 5480990; US 5462725; US 5428139; US 5385893; US 5739294; US 5750660; US 5834456; Hnatowich et al (1983) J. Immunol. Methods 65:147-157; Meares et al (1984) Anal. Biochem. 142:68-78; Mirzadeh et al (1990) Bioconjugate Chem. 1:59-65; Meares et al (1990) J. Cancer1990, Suppl. 10:21-26; Izard et al (1992) Bioconjugate Chem. 3:346-350; Nikula et al (1995) Nucl. Med. Biol. 22:387-90; Camera et al (1993) Nucl. Med. Biol. 20:955-62; Kukis et al (1998) J. Nucl. Med. 39:2105-2110; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Camera et al (1994) J. Nucl. Med. 21:640-646; Ruegg et al (1990) Cancer Res. 50:4221-4226; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Lee et al (2001) Cancer Res. 61:4474-4482; Mitchell, et al (2003) J. Nucl. Med. 44:1105-1112; Kobayashi et al (1999) Bioconjugate Chem. 10:103-111; Miederer et al (2004) J. Nucl. Med. 45:129-137; DeNardo et al (1998) Clinical Cancer Research 4:2483-90; Blend et al (2003) Cancer Biotherapy & Radiopharmaceuticals 18:355-363; Nikula et al (1999) J. Nucl. Med. 40:166-76; Kobayashi et al (1998) J. Nucl. Med. 39:829-36; Mardirossian et al (1993) Nucl. Med. Biol. 20:65-74; Roselli et al (1999) Cancer Biotherapy & Radiopharmaceuticals, 14:209-20.

(b) Fluorescent labels such as rare earth chelates (europium chelates), fluorescein types including FITC, 5-carboxyfluorescein, 6-carboxy fluorescein; rhodamine types including TAMRA; dansyl;

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Lissamine; cyanines; phycoerythrins; Texas Red; and analogs thereof. The fluorescent labels can be conjugated to antibodies using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescent dyes and fluorescent label reagents include those which are commercially available from Invitrogen/Molecular Probes (Eugene, OR) and Pierce Biotechnology, Inc. (Rockford, IL).

(c) Various enzyme-substrate labels are available or disclosed (US 4275149). The enzyme generally catalyzes a chemical alteration of a chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; US 4737456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al (1981) "Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay", in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic Press, New York, 73:147-166.

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRP) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review, see US 4275149 and US 4318980.

A label may be indirectly conjugated with an amino acid side chain, an acitivated amino acid side chain, a cysteine engineered antibody, and the like. For example, the antibody can be conjugated with

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biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin or streptavidin, or vice versa. Biotin binds selectively to streptavidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the polypeptide variant, the polypeptide variant is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten polypeptide variant (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the polypeptide variant can be achieved (Hermanson, G. (1996) in Bioconjugate Techniques Academic Press, San Diego).

The antibody of the present invention may be employed in any known assay method, such as ELISA, competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Zola, (1987) Monoclonal Antibodies: A Manual of Techniques, pp.147-158, CRC Press, Inc.).

A detection label may be useful for localizing, visualizing, and quantitating a binding or recognition event. The labelled antibodies of the invention can detect cell-surface receptors. Another use for detectably labelled antibodies is a method of bead-based immunocapture comprising conjugating a bead with a fluorescent labelled antibody and detecting a fluorescence signal upon binding of a ligand. Similar binding detection methodologies utilize the surface plasmon resonance (SPR) effect to measure and detect antibody-antigen interactions.

Detection labels such as fluorescent dyes and chemiluminescent dyes (Briggs et al (1997) "Synthesis of Functionalised Fluorescent Dyes and Their Coupling to Amines and Amino Acids," J. Chem. Soc., Perkin-Trans. 1:1051-1058) provide a detectable signal and are generally applicable for labelling antibodies, preferably with the following properties: (i) the labelled antibody should produce a very high signal with low background so that small quantities of antibodies can be sensitively detected in both cell-free and cell-based assays; and (ii) the labelled antibody should be photostable so that the fluorescent signal may be observed, monitored and recorded without significant photo bleaching. For applications involving cell surface binding of labelled antibody to membranes or cell surfaces, especially live cells, the labels preferably (iii) have good water-solubility to achieve effective conjugate concentration and detection sensitivity and (iv) are non-toxic to living cells so as not to disrupt the normal metabolic processes of the cells or cause premature cell death.

Direct quantification of cellular fluorescence intensity and enumeration of fluorescently labelled events, e.g. cell surface binding of peptide-dye conjugates may be conducted on an system (FMAT® 8100 HTS System, Applied Biosystems, Foster City, Calif.) that automates mix-and-read, non-radioactive assays with live cells or beads (Miraglia, "Homogeneous cell- and bead-based assays for high throughput screening using fluorometric microvolume assay technology", (1999) J. of Biomolecular Screening 4:193-

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204). Uses of labelled antibodies also include cell surface receptor binding assays, inmmunocapture assays, fluorescence linked immunosorbent assays (FLISA), caspase-cleavage (Zheng, "Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo", (1998) Proc. Natl. Acad. Sci. USA 95:618-23; US 6372907), apoptosis (Vermes, "A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V" (1995) J. Immunol. Methods 184:39-51) and cytotoxicity assays. Fluorometric microvolume assay technology can be used to identify the up or down regulation by a molecule that is targeted to the cell surface (Swartzman, "A homogeneous and multiplexed immunoassay for high-throughput screening using fluorometric microvolume assay technology", (1999) Anal. Biochem. 271:143-51).

Labelled antibodies of the invention are useful as imaging biomarkers and probes by the various methods and techniques of biomedical and molecular imaging such as: (i) MRI (magnetic resonance imaging); (ii) MicroCT (computerized tomography); (iii) SPECT (single photon emission computed tomography); (iv) PET (positron emission tomography) Chen et al (2004) Bioconjugate Chem. 15:41-49; (v) bioluminescence; (vi) fluorescence; and (vii) ultrasound. Immunoscintigraphy is an imaging procedure in which antibodies labeled with radioactive substances are administered to an animal or human patient and a picture is taken of sites in the body where the antibody localizes (US 6528624). Imaging biomarkers may be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers may be of several types: Type 0 are natural history markers of a disease and correlate longitudinally with known clinical indices, e.g. MRI assessment of synovial inflammation in rheumatoid arthritis; Type I markers capture the effect of an intervention in accordance with a mechanism-of-action, even though the mechanism may not be associated with clinical outcome; Type II markers function as surrogate endpoints where the change in, or signal from, the biomarker predicts a clinical benefit to "validate" the targeted response, such as measured bone erosion in rheumatoid arthritis by CT. Imaging biomarkers thus can provide pharmacodynamic (PD) therapeutic information about: (i) expression of a target protein, (ii) binding of a therapeutic to the target protein, i.e. selectivity, and (iii) clearance and half-life pharmacokinetic data. Advantages of in vivo imaging biomarkers relative to lab-based biomarkers include: non-invasive treatment, quantifiable, whole body assessment, repetitive dosing and assessment, i.e. multiple time points, and potentially transferable effects from preclinical (small animal) to clinical (human) results. For some applications, bioimaging supplants or minimizes the number of animal experiments in preclinical studies.

Peptide labelling methods are well known. See Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, (1997) Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means

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(1990) Bioconjugate Chem. 1:2; Glazer et al (1975) Chemical Modification of Proteins. Laboratory Techniques in Biochemistry and Molecular Biology (T. S. Work and E. Work, Eds.) American Elsevier Publishing Co., New York; Lundblad, R. L. and Noyes, C. M. (1984) Chemical Reagents for Protein Modification, Vols. I and II, CRC Press, New York; Pfleiderer, G. (1985) "Chemical Modification of Proteins", Modern Methods in Protein Chemistry, H. Tschesche, Ed., Walter DeGryter, Berlin and New York; and Wong (1991) Chemistry of Protein Conjugation and Cross-linking, CRC Press, Boca Raton, Fla.); De Leon-Rodriguez et al (2004) Chem.Eur. J. 10:1149-1155; Lewis et al (2001) Bioconjugate Chem. 12:320-324; Li et al (2002) Bioconjugate Chem. 13:110-115; Mier et al (2005) Bioconjugate Chem. 16:240-237.

Peptides and proteins labelled with two moieties, a fluorescent reporter and quencher in sufficient proximity undergo fluorescence resonance energy transfer (FRET). Reporter groups are typically fluorescent dyes that are excited by light at a certain wavelength and transfer energy to an acceptor, or quencher, group, with the appropriate Stokes shift for emission at maximal brightness. Fluorescent dyes include molecules with extended aromaticity, such as fluorescein and rhodamine, and their derivatives. The fluorescent reporter may be partially or significantly quenched by the quencher moiety in an intact peptide. Upon cleavage of the peptide by a peptidase or protease, a detectable increase in fluorescence may be measured (Knight, C. (1995) "Fluorimetric Assays of Proteolytic Enzymes", Methods in Enzymology, Academic Press, 248:18-34).

The labelled antibodies of the invention may also be used as an affinity purification agent. In this process, the labelled antibody is immobilized on a solid phase such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing the antigen to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the antigen to be purified, which is bound to the immobilized polypeptide variant. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the antigen from the polypeptide variant.

Labelling reagents typically bear reactive functionality which may react (i) directly with a cysteine thiol of a cysteine engineered antibody to form the labelled antibody, (ii) with a linker reagent to form a linker-label intermediate, or (iii) with a linker antibody to form the labelled antibody. Reactive functionality of labelling reagents include: maleimide, haloacetyl, iodoacetamide succinimidyl ester (e.g. NHS, N-hydroxysuccinimide), isothiocyanate, sulfonyl chloride, 2,6-dichlorotriazinyl, pentafluorophenyl ester, and phosphoramidite, although other functional groups can also be used.

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An exemplary reactive functional group is N-hydroxysuccinimidyl ester (NHS) of a carboxyl group substituent of a detectable label, e.g. biotin or a fluorescent dye. The NHS ester of the label may be preformed, isolated, purified, and/or characterized, or it may be formed in situ and reacted with a nucleophilic group of an antibody. Typically, the carboxyl form of the label is activated by reacting with some combination of a carbodiimide reagent, e.g. dicyclohexylcarbodiimide, diisopropylcarbodiimide, or a uronium reagent, e.g. TSTU (O-(N-Succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, HBTU (O-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), or HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), an activator, such as 1-hydroxybenzotriazole (HOBt), and N-hydroxysuccinimide to give the NHS ester of the label. In some cases, the label and the antibody may be coupled by in situ activation of the label and reaction with the antibody to form the label-antibody conjugate in one step. Other activating and coupling reagents include TBTU (2-(1H-benzotriazo-1-yl)-1-1,3,3-tetramethyluronium hexafluorophosphate), TFFH (N,N',N",N'"-tetramethyluronium 2-fluoro-hexafluorophosphate), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline), DCC (dicyclohexylcarbodiimide); DIPCDI (diisopropylcarbodiimide), MSNT (1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole, and aryl sulfonyl halides, e.g. triisopropylbenzenesulfonyl chloride.

Albumin binding peptide-Fab compounds of the invention:

In one aspect, the antibody of the invention is fused to an albumin binding protein. Plasma-protein binding can be an effective means of improving the pharmacokinetic properties of short lived molecules. Albumin is the most abundant protein in plasma. Serum albumin binding peptides (ABP) can alter the pharmacodynamics of fused active domain proteins, including alteration of tissue uptake, penetration, and diffusion. These pharmacodynamic parameters can be modulated by specific selection of the appropriate serum albumin binding peptide sequence (US 20040001827). A series of albumin binding peptides were identified by phage display screening (Dennis et al. (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" J Biol Chem. 277:35035-35043; WO 01/45746). Compounds of the invention include ABP sequences taught by: (i) Dennis et al (2002) J Biol Chem. 277:35035-35043 at Tables III and IV, page 35038; (ii) US 20040001827 at [0076] SEQ ID NOS: 9-22; and (iii) WO 01/45746 at pages 12-13, all of which are incorporated herein by reference. Albumin Binding (ABP)-Fabs are engineered by fusing an albumin binding peptide to, for example, the C-terminus of Fab heavy chain in 1:1 stoichiometric ratio (1 ABP / 1 Fab). It was shown that association of these ABP-Fabs with albumin increased antibody half life by more than 25 fold in rabbits and mice. The above described reactive Cys residues can therefore be introduced in these ABP-Fabs and used for site-specific conjugation with cytotoxic drugs followed by in vivo animal studies.

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Exemplary albumin binding peptide sequences include, but are not limited to, the amino acid sequences listed in SEQ ID NOS:80-84.

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	SEQ ID NO:80
QRLMEDICLPRWGCLWEDDF	SEQ ID NO:81
QRLIEDICLPRWGCLWEDDF	SEQ ID NO:82
RLIEDICLPRWGCLWEDD	SEQ ID NO:83
DICLPRWGCLW	SEQ ID NO:84

Antibody-Drug Conjugates

In another aspect, the invention provides immunoconjugates, or antibody-drug conjugates (ADC), comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). In another aspect, the invention further provides methods of using the immunoconjugates. In one aspect, an immunoconjugate comprises any of the above anti-STEAP-1 antibodies covalently attached to a cytotoxic agent or a detectable agent.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4,975,278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., (1986) supra). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) Jour. of the 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

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calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas) and cAC10 (specific to CD30 on hematological malignancies) (Doronina et al (2003) Nature Biotechnology 21(7):778-784) and are under therapeutic development.

Chemotherapeutic agents useful in the generation of immunoconjugates are described herein. Enzymatically active toxins and fragments thereof that 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, e.g., WO 93/21232 published October 28, 1993. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as

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N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al (1987) Science, 238:1098. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (WO94/11026).

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

Maytansine and maytansinoids

In some embodiments, the immunoconjugate comprises an antibody (full length or fragments) of the invention conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

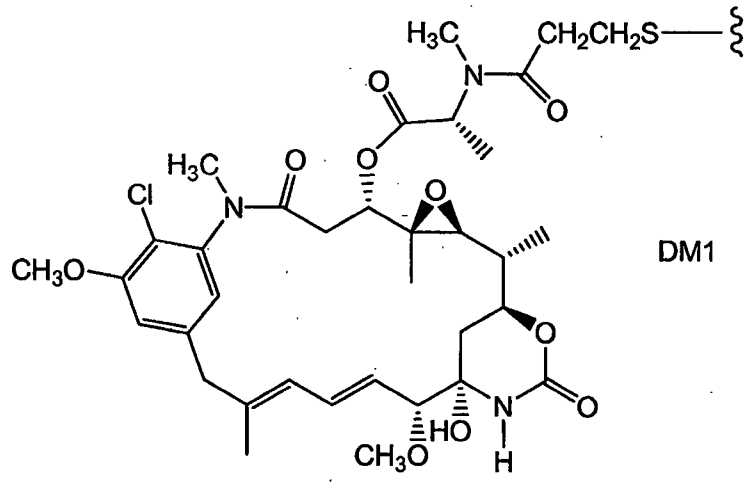
Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods.

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Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (US 4256746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (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

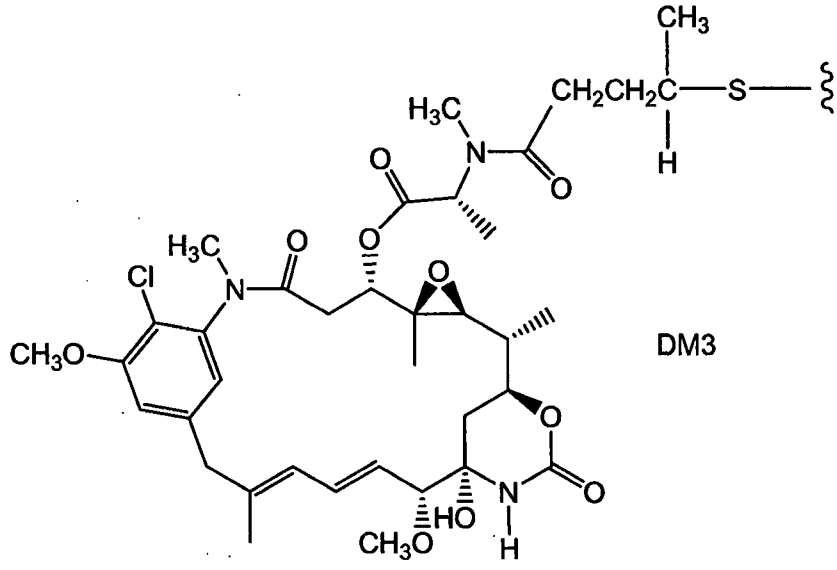
Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (US 4424219) (prepared by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (US 4450254) (prepared from Nocardia); C-15-hydroxy/acyloxy (US 4364866) (prepared by the conversion of maytansinol by Streptomyces); C-15-methoxy (US Pat. Nos. 4313946 and 4315929) (isolated from Trewia nudlflora); C-18-N-demethyl (US Pat. Nos. 4362663 and 4322348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (US 4371533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, having the structures:

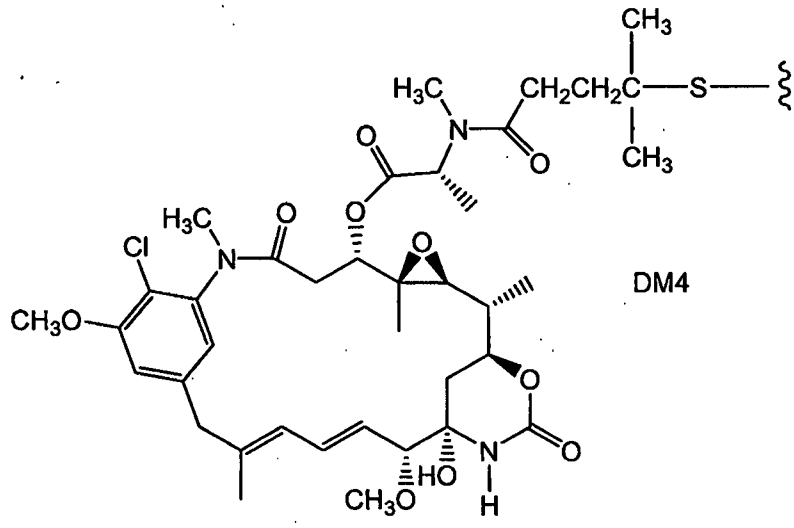


DM1

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DM3

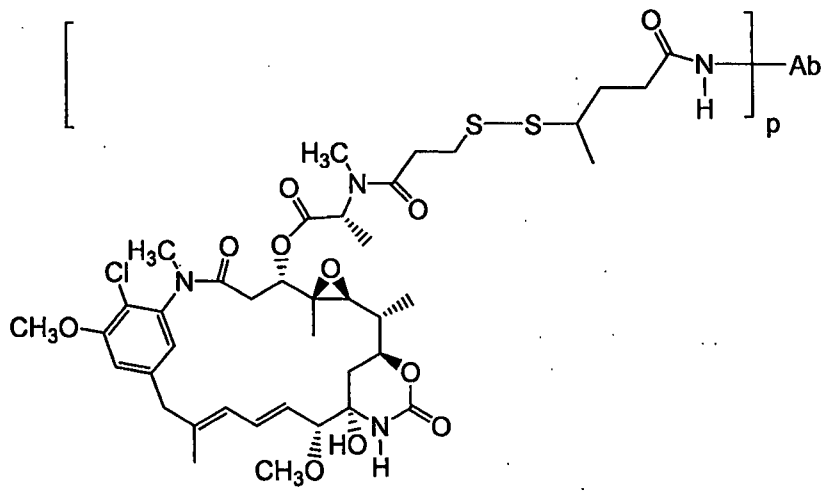


DM4

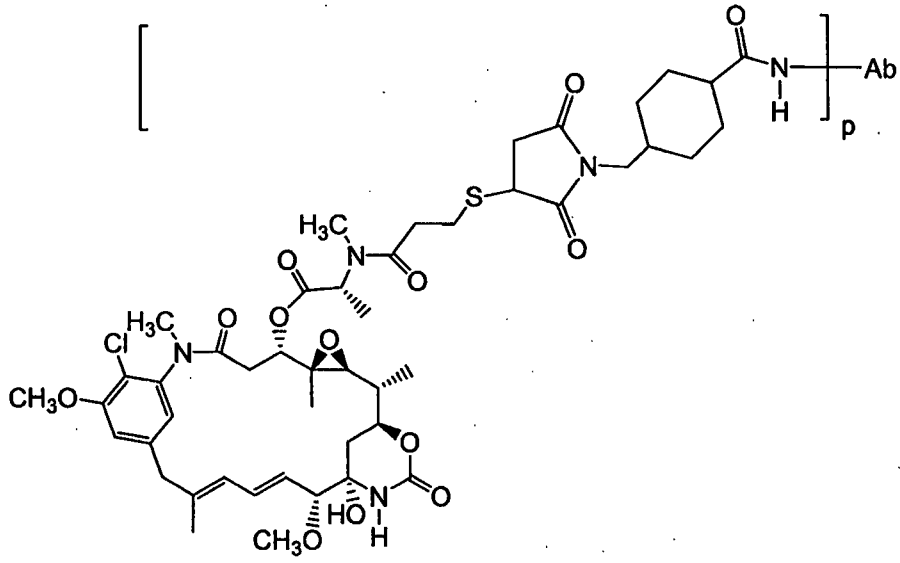
wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody drug conjugate. HERCEPTIN® (trastuzumab) linked by SMCC to DM1 has been reported (WO 2005/037992, which is expressly incorporated herein by reference in its entirety). An antibody drug conjugate of the present invention may be prepared according to the procedures disclosed therein.

Other exemplary maytansinoid antibody drug conjugates have the following structures and abbreviations, (wherein Ab is antibody and p is 1 to about 8):

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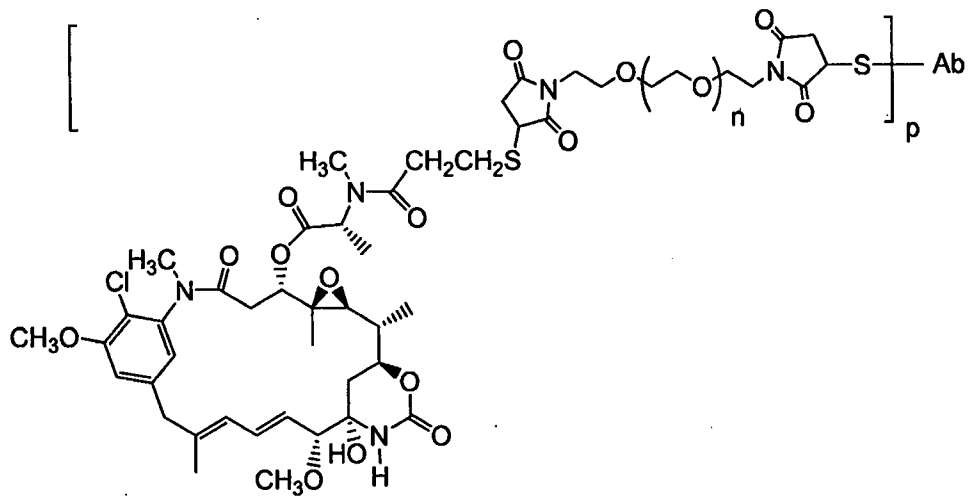
Ab -SPP-DM1



Ab-SMCC-DM1

Exemplary antibody drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:

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where Ab is antibody; n is 0, 1, or 2; and p is 1, 2, 3, or 4.

Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020; 5,416,064; 6,441163 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 immunoconjugates 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. Chari et al., Cancer Research 52:127-131 (1992) describe immunoconjugates 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 3 x 10^5 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.

Anti-STEAP-1 antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed,

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for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent Nos. 5208020, 6441163, or EP Patent 0 425 235 B1, Chari et al., Cancer Research 52:127-131 (1992), and US 2005/0169933 A1, the disclosures of which are hereby expressly incorporated by reference. Antibody-maytansinoid conjugates comprising the linker component SMCC may be prepared as disclosed in U.S. Patent Application No. 11/141344, filed 31 May 2005, "Antibody Drug Conjugates and Methods". The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents. Additional linking groups are described and exemplified herein.

Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 (1978)) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

In one embodiment, any of the antibodies of the invention (full length or fragment) is conjugated to one or more maytansinoid molecules. In one embodiment of the immunoconjugate, the cytotoxic agent D, is a maytansinoid DM1. In one embodiment of the immunoconjugate, the linker is selected from the group consisting of SPDP, SMCC, IT, SPDP, and SPP.

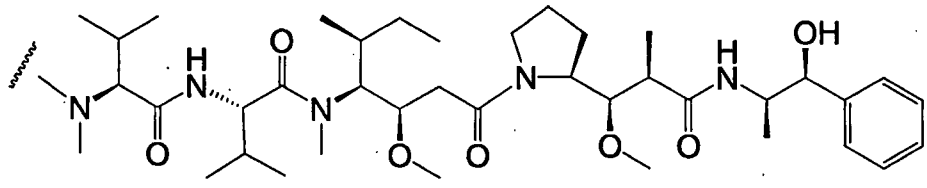
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Auristatins and dolostatins

In some embodiments, the immunoconjugate comprises an antibody of the invention conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (US Patent Nos. 5635483; 5780588). 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 (US 5663149) 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).

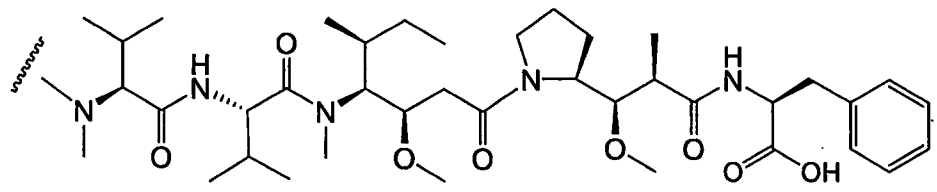
Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

An exemplary auristatin embodiment is MMAE (wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate).



MMAE

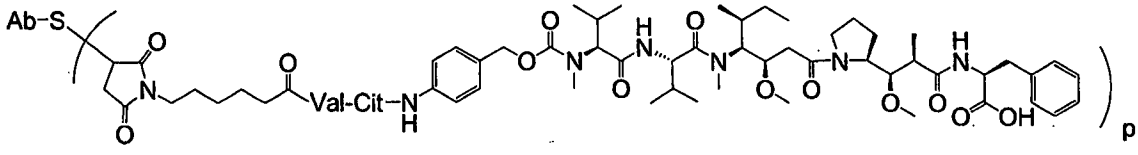
Another exemplary auristatin embodiment is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate (US 2005/0238649):



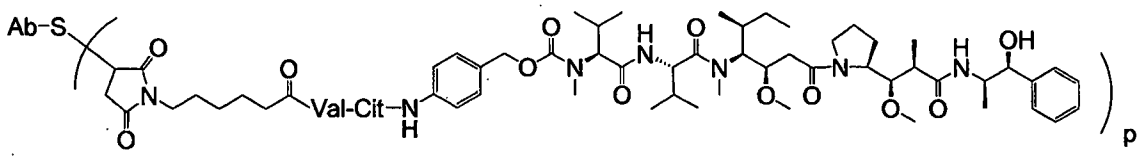
MMAF

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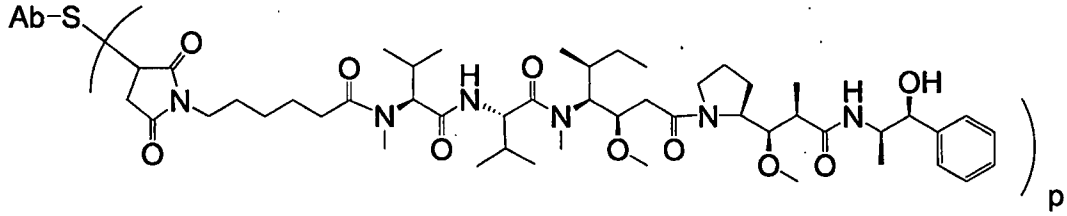
Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):



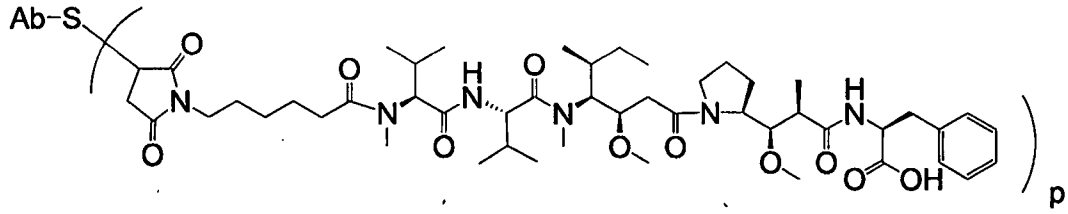
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

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. Schröder and K. Lübke, "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: US 5635483; US 5780588; 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.

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Calicheamicin

In other embodiments, the immunoconjugate comprises an antibody of the invention conjugated to one or more calicheamicin molecules. 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. patents 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, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman et al., Cancer Research 53:3336-3342 (1993), 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.

Other cytotoxic agents

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. patents 5,053,394, 5,770,710, as well as esperamicins (U.S. patent 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

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

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 At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic

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resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

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 tc^{99m} or I^{123}, .Re^{186}, Re^{188} and In^{111} 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.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

Preparation of antibody drug conjugates:

In the antibody drug conjugates (ADC) of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), e.g. about 1 to about 20 drug moieties per antibody, through a linker (L). In one

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embodiment, the number of drug moieties (D) per antibody is from about 1 to about 5, alternatively, from about 2 to about 6, alternatively, from about 2 to about 5, alternatively from about 3 to about 4 drug moieties per antibody. Because the number of drug moieties per antibody is typically an average number over all conjugates in a population of an antibody drug conjugate, the number of drug moieties per antibody may not be a whole number. The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent, to form Ab-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of an antibody. Additional methods for preparing ADC are described herein.

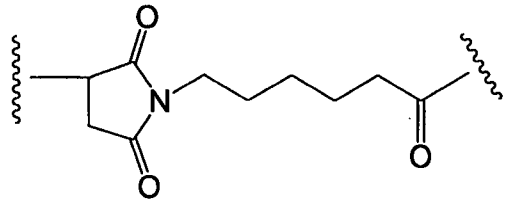
$$Ab–(L–D)_p \qquad \text{Formula I}$$

The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC'), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). In on embodiment, the linker is valine-citrullin-p-aminobenzyloxycaronyl ("vc-PAB"). Additional linker components are known in the art and some are described herein.

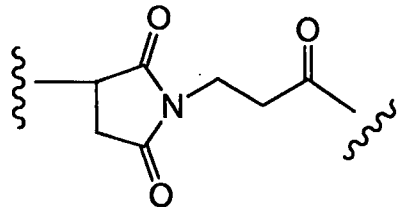
In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Exemplary linker component structures are shown below (wherein the wavy line indicates sites of covalent attachment to other components of the ADC):

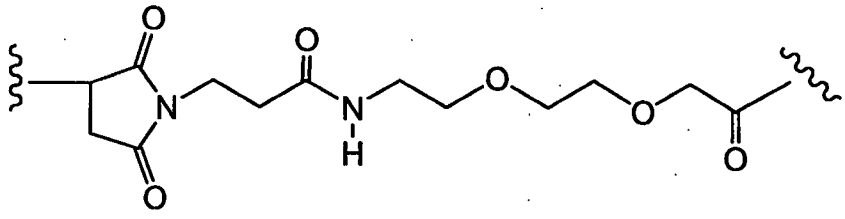
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MC

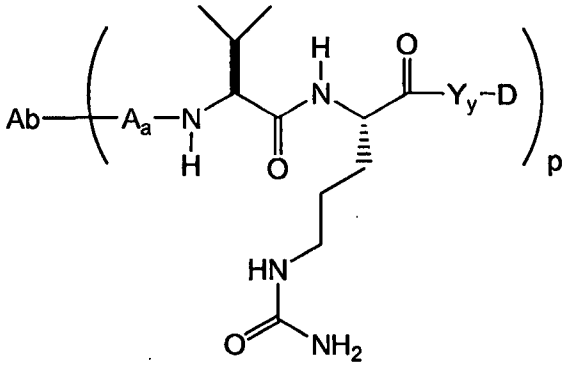


MP

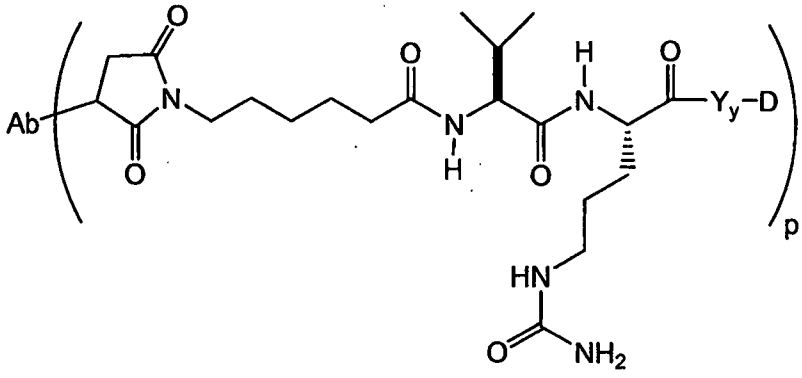


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Additional exemplary linker components and abbreviations include (wherein the antibody (Ab) and linker are depicted, and p is 1 to about 8):

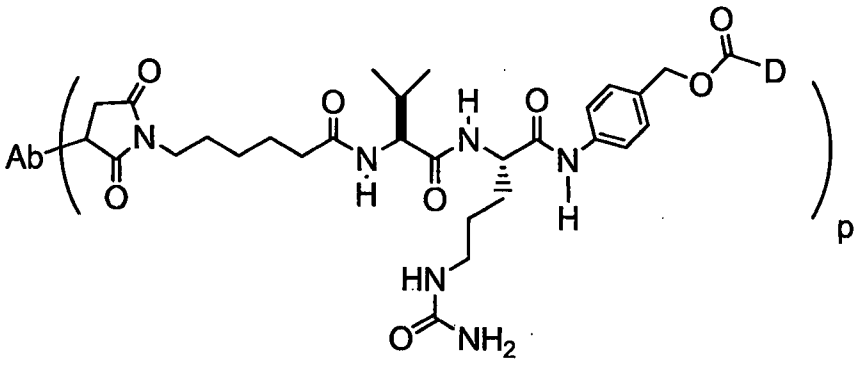


Val-cit



MC-val-cit

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MC-val-cit-PAB

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by introducing one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody drug conjugates of the invention may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic subsituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either glactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first

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amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such aldehyde can be reacted with a drug moiety or linker nucleophile.

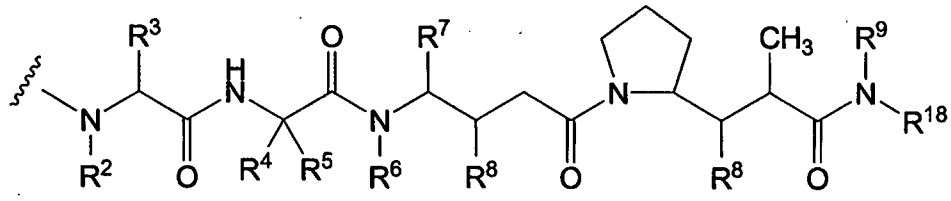
Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Methods for the conjugation of linker-drug moieties to cell-targeted proteins such as antibodies, immunoglobulins or fragments thereof are found, for example, in US5,208,020; US6,441,163; WO2005037992; WO2005081711; and WO2006/034488, all of which are hereby expressly incorporated by reference in their entirety.

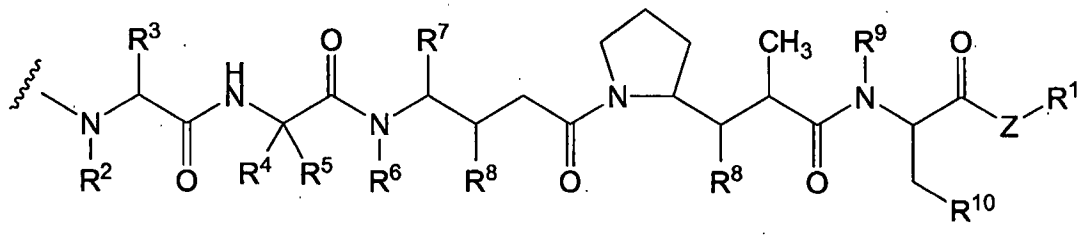
Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

In one embodiment of the immunoconjugate, the cytotoxic agent, D, is an auristatin of formula D_E or D_F



D_E



D_F

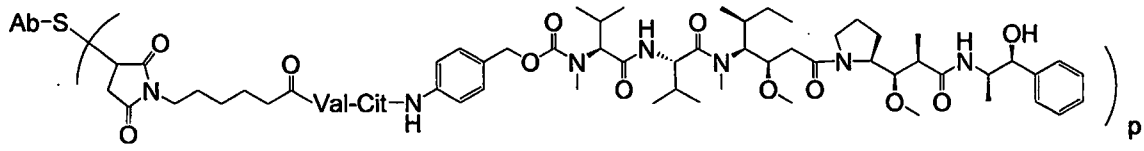
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and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-CH_3$; and R^{18} is $-C(R^8)_2-C(R^8)_2$–aryl; and

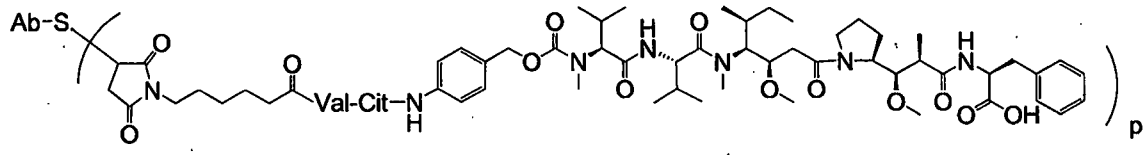
(d) p ranges from about 1 to 8.

The following embodiments are further provided for any of the above immunoconjugates. In one embodiment, an immunoconjugate has in vitro or in vivo cell killing activity. In one embodiment, the linker is attached to the antibody through a thiol group on the antibody. In one embodiment, the linker is cleavable by a protease. In one embodiment, the linker comprises a val-cit dipeptide. In one embodiment, the linker comprises a p-aminobenzyl unit. In one embodiment, the p-aminobenzyl unit is disposed between the drug and a protease cleavage site in the linker. In one embodiment, the p-aminobenzyl unit is p-aminobenzyloxycarbonyl (PAB). In one embodiment, the linker comprises 6-maleimidocaproyl. In one embodiment, the 6-maleimidocaproyl is disposed between the antibody and a protease cleavage site in the linker. The above embodiments may occur singly or in any combination with one another.

In one embodiment, the drug is selected from MMAE and MMAF. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 2 to about 5. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 1 to about 6, from about 2 to about 5, from about 2 to about 6, from about 2 to about 4, from about 2 to about 3, from about 3 to about 4, from about 3 to about 5, from about 3 to about 6, or from about 4 to about 6.

Labelled antibody imaging methods:

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In another embodiment of the invention, cysteine engineered antibodies may be labelled through the cysteine thiol with radionuclides, fluorescent dyes, bioluminescence-triggering substrate moieties, chemiluminescence-triggering substrate moieties, enzymes, and other detection labels for imaging experiments with diagnostic, pharmacodynamic, and therapeutic applications. Generally, the labelled cysteine engineered antibody, i.e. "biomarker" or "probe", is administered by injection, perfusion, or oral ingestion to a living organism, e.g. human, rodent, or other small animal, a perfused organ, or tissue sample. The distribution of the probe is detected over a time course and represented by an image.

Articles of Manufacture:

In another embodiment of the invention, an article of manufacture, or "kit", containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds an antibody-drug conjugate (ADC) composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an ADC. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Pharmaceutical compositions:

In one aspect, a pharmaceutical composition is provided comprising any of the above immunoconjugates and a pharmaceutically acceptable carrier. In one aspect, a method of treating a prostate, lung, colon, bladder, or ovarian cell proliferative disorder, or Ewing's sarcoma is provided, wherein the method comprises administering to an individual the pharmaceutical composition. In one embodiment, the prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma cell proliferative disorder is a metastasis of a primary prostate, lung, colon, bladder, or ovarian cancer or Ewing's sarcoma. In one embodiment, the cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a cell.

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In one aspect, a method of inhibiting cell proliferation is provided, wherein the method comprises exposing a cell to any of the above immunoconjugates under conditions permissive for binding of the immunoconjugate to STEAP-1. In one embodiment, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma is a tumor cell. In one embodiment, the tumor cell is a prostate, lung, colon, bladder, or ovarian tumor cell or Ewing's sarcoma celll of a mammal experiencing or suspected of experiencing prostate, lung, colon, bladder cell or Ewing's sarcoma proliferative disorder including, but not limited to, a metastasis of a primary prostate, lung, colon, bladder cell cancer tumor or Ewing's sarcoma tumor. In one embodiment, the prostate, lung, colon, bladder cell or Ewing's sarcoma is a xenograft. In one embodiment, the exposing takes place in vitro. In one embodiment, the exposing takes place in vivo.

In one aspect, a method of using the anti-STEAP-1 antibody of the invention is provided to assay serum soluble STEAP-1 in a mammal experiencing prostate lung or colon cell proliferative disorder (or metastasis of a primary incidence of such disorder), measuring clinical progression or regression of the diseases, or assess tumor burden or relapse.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the amino acid sequence of human STEAP-1 (SEQ ID NO:1) aligned with STEAP-1 from mouse and cynomolgus monkey (cyno) (SEQ ID NOs:2 and 3, respectively). Extracellular domains 1, 2, and 3 are labeled and marked by shaded boxes.

Figures 2A-2B: **Figure 2A** depicts the amino acid sequence of the light chain variable region of murine 120.545 anti-STEAP-1 antibody aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human subgroup III sequence. The CDRs are boxed (CDR-L1, CDR-L2, and CDR-L3). The sequences bracketing the CDRs are the framework sequences (FR-L1 to FR-L4). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs. **Figure 2B** depicts the amino acid sequence of the heavy chain variable region of murine anti-STEAP-1 antibody (120.545) aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human kappa I sequence. Humanized variants 24, 37, 48, 67, and 37/48, 67, 71, and 78 were prepared by making the following amino acid changes: A24V, V37I, V48M, F67I, and L78F in the heavy chain of the 120 graft antibody. The CDRs are boxed. The FR-H1, FR-H2, FR-H3 and FR-H4 sequences bracket the CDRs (CDR-H1, CDR-H2, and CDR-H3). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs.

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Figures 3A and 3B show exemplary acceptor human variable heavy (VH) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows, where the FR SEQ ID NOs are listed in the order FR-H1, FR-H2, FR-H3, FR-H4:

- human VH subgroup I consensus framework "A" minus Kabat CDRs (SEQ ID NOs:26, 27, 28, 29).

- human VH subgroup I consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:30, 31, 28, 29; SEQ ID NOs:30, 31, 32, 29; and SEQ ID NOs:30, 31, 33, 29).

- human VH subgroup II consensus framework "A" minus Kabat CDRs (SEQ ID NOs:34, 35, 36, 29).

- human VH subgroup II consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:37, 38, 36, 29; SEQ ID NOs:37, 38, 39, 29; and SEQ ID NOs:37, 38, 40, 29).

- human VH subgroup III consensus framework "A" minus Kabat CDRs (SEQ ID NOs:41, 42, 43, 29).

- human VH subgroup III consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 43, 29; SEQ ID NOs:44, 45, 46, 29; and SEQ ID NOs:44, 45, 46, 29).

- human VH acceptor 1 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 49, 29).

- human VH acceptor frameworks "B" and "C" minus extended hypervariable regions (SEQ ID NOs:44, 45, 49, 29; and SEQ ID NOs:44, 45, 50, 29).

- human VH acceptor 2 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 51, 29).

- human VH acceptor 2 framework "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 51, 29; SEQ ID NOs:44, 45, 52, 29; and SEQ ID NOs:44, 45, 53, 29).

Figures 4A and 4B show exemplary acceptor human variable light (VL) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows:

- human VL kappa subgroup I-1 consensus framework (κv1-1): SEQ ID NOs:54, 55, 56, 57

- human VL kappa subgroup I consensus framework (κv1): SEQ ID NOs:54, 58, 56, 57

- human VL kappa subgroup II consensus framework (κv2): SEQ ID NOs:58, 59, 60, 57

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- human VL kappa subgroup III consensus framework (κv3): SEQ ID NOs:61, 62, 63, 57

- human VL kappa subgroup IV consensus framework (κv4): SEQ ID NOs:64, 65, 66, 57.

Figure 5 depicts alignments of native sequence human IgG Fc region sequences, humIgG1 (non-A allotype, SEQ ID NO:85; and A allotype, where the amino acid sequence SREEM within SEQ ID NO:85 is changed to SRDEL), humIgG2 (SEQ ID NO:86), humIgG3 (SEQ ID NO:87) and humIgG4 (SEQ ID NO:88) with differences between the sequences marked with asterisks. Numbers above the sequences represent the EU numbering system. An exemplary kappa constant region is also shown.

Figures 6A-6D depicts a FACS analysis normalized for the level of display of each antibody or variant on phage. Figure 6A shows FACS shifts for four exemplary antibodies. Figure 6B is an alignment of FACS shifts for several antibodies as indicated in the figure and in Example 1. Figures 6C and 6D are FACS shift alignments after normalization for phage display levels.

Figures 7A-7F graphically depict FACS analyses showing binding of anti-STEAP-1 that the anti-STEAP-1 murine, chimera and humanized version 24 antibodies to human STEAP-1 express on the cell surface. Figures 7A-7C indicate that anti-STEAP-1 murine 120, chimera 120 and humanized 120v.24 bind human and cynomolgus monkey STEAP-1, but not mouse STEAP-1. Figures 7D-7F are FACS plots showing binding of murine 120, 120 chimera, and humanized 120v.24 (clone 67) to human STEAP-1 expressed on the cell surface. Exogenous STEAP-1 was stably expressed in 293 cells (designated LB50 cells) and PC3 cells (designated PS5.4 cells) (Figures 12A and 12B), and endogenously expressed in LNCaP BR cells (Figure 12C).

Figures 8A and 8B. Figure 8A is a graph showing that administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. See Example 4. **Figure 8B** is a graph showing that single dose administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg); and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a LNCaP cell xenograft prostate tumor model. See Example 4.

Figure 9 is a graph showing that administration of anti-STEAP-1antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE) at 3 mg/kg, or anti-STEAP-1 120 chimera-MC-MMAF (abbreviated anti-STEAP mcMMAF) at 6 mg/kg, was shown to be efficacious in a prostate cancer xenograft model of castrated SCID-beige mice transplanted with LNCaP cells. See Example 4.

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Figure 10 is a graph showing that administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE (at 3 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. See Example 4.

Figure 11 is a graph showing that administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, humanized anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. See Example 4.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Isolated antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are further provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as a tumor or cancer. In certain embodiments, STEAP-1 is expressed in tumor or cancer of prostate, lung, or colon tissue. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1, e.g., STEAP-1 expressed on the cell surface. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1 expression on the surface of normal and/or tumor or cancer cells of protate, lung or colon tissue.

Polynucleotides encoding anti-STEAP-1 antibodies are provided. Vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided, and host cells comprising such vectors are provided. Compositions, including pharmaceutical formulations, comprising any one or more of the polynucleotides, anti-STEAP-1 antibodies, or immunoconjugates of the invention are also provided.

Methods of treating a cell proliferative disorder, including but not limited to tumor or cancer, with an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, treatment of tumor or cancer in prostate, lung or colon of a mammal. Methods of detecting STEAP-1 expression on a tissue cell using an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, detection of STEAP-1 expression on, as a non-limiting example, a normal cell, tumor cell, or cancer cell of prostate, lung, or colon cell.

General Techniques

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The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds., (2003)); the series Methods in Enzymology (Academic Press, Inc.): Pcr 2: A Practical Approach (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture (R. I. Freshney, ed. (1987)); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney), ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V. T. DeVita et al., eds., J.B. Lippincott Company, 1993).

DEFINITIONS AND ABBREVIATIONS

Definitions

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within

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recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is separated from at least one other nucleic acid molecule with which it is ordinarily associated, for example, in its natural environment. An isolated nucleic acid molecule further includes a nucleic acid molecule contained in cells that ordinarily express the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Purified" means that a molecule is present in a sample at a concentration of at least 95% by weight, or at least 98% by weight of the sample in which it is contained.

The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and

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episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors," or simply, "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"),

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P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

"Oligonucleotide," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

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where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "STEAP-1," as used herein, refers to any native STEAP-1 from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed STEAP-1 as well as any form of STEAP-1 that results from processing in the cell. The term also encompasses naturally occurring variants of STEAP-1, e.g., splice variants, allelic variants, and isoforms. The amino acid sequence of human STEAP-1 is depicted in Figure 1 (SEQ ID NO:1). In one embodiment, STEAP-1 is expressed on the cell surface, such as on the surface of a normal prostate, lung or colon cell, and has increased expression in prostate, lung or colon cancer cells or metastases of such cancer cells. Figure 1 also depicts the amino acid sequence of STEAP-1 from mouse and cynomolgus monkey (SEQ ID NOs:2 and 3, respectively).

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having similar structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, monovalent antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized and/or affinity matured.

The term "anti-STEAP-1 antibody" or "an antibody that binds to STEAP-1" refers to an antibody that is capable of binding STEAP-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting STEAP-1. Preferably, the extent of binding of an anti-STEAP-1 antibody to an unrelated, non-STEAP-1 protein is less than about 10% of the binding of the antibody to

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STEAP-1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to STEAP-1 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an anti-STEAP-1 antibody binds to an epitope of STEAP-1 that is conserved among STEAP-1 from different species.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

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

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

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (2000). An antibody may be

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part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

"Antibody fragments" comprise only a portion of an intact antibody, wherein the portion retains at least one, and as many as most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half life substantially similar to an intact antibody. For example, such an antibody fragment may comprise on antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

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

"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments

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differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO93/1161; Hudson et al. (2003) Nat. Med. 9:129-134; and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al. (2003) Nat. Med. 9:129-134.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast

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to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler et al., Nature, 256: 495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage display technologies (see, e.g., Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132(2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893; WO96/34096; WO96/33735; WO91/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks et al., Bio.Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996) and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody

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is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six hypervariable regions, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. Xu et al. (2000) Immunity 13:37-45; Johnson and Wu (2003) in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, NJ). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. Hamers-Casterman et al. (1993) Nature 363:446-448; Sheriff et al. (1996) Nature Struct. Biol. 3:733-736.

A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the

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structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions. The HVR-H1, HVR-H2, and HVR-H3 hypervariable regions of the humanized anti-STEAP-1 120v.24 antibodies of the invention are H26-H35A, H49-H6, and H95-H102 using Kabat numbering. The HVR-L1, HVR-L2, and HVR-L3 hypervariable regions of the humanized anti-STEP-1 120v.24 antibodies of the invention are L24-34, L50-56, and L89-97 using Kabat numbering. As used herein, the terms "HVR" and "CDR" are used interchangeably.

"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For

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example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

An "affinity matured" antibody is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In one embodiment, an affinity matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. Bio/Technology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of HVR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds. Certain blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

An "agonist antibody," as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor

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FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, e.g., Ghetie 1997, Hinton 2004). Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates administered with the Fc variant polypeptides.

WO00/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. The content of that patent publication is specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source, e.g., from blood.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or Presta U.S. Patent No. 6,737,056 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

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"Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed.

Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US Patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

The term "Fc region-comprising polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin, which comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the polypeptide or by recombinant engineering the nucleic acid encoding the polypeptide. Accordingly, a composition comprising a polypeptide having an Fc region according to this invention can comprise polypeptides with K447, with all K447 removed, or a mixture of polypeptides with and without the K447 residue.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferably those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may be 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

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In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat et al., supra. In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: EVQLVESGGGLVQPGGSLRLSCAAS (FR-H1, SEQ ID NO:21)-HVR-H1-WVRQAPGKGLEWV (FR-H2, SEQ ID NO:22)-HVR-H2-RFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR (FR-H3, SEQ ID NO:23)-HVR-H3-WGQGTLVTVSS (FR-H4, SEQ ID NO:24).

A "VL subgroup I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat et al., supra. In one embodiment, the VH subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: DIQMTQSPSSLSASVGDRVTITC (FR-L1, SEQ ID NO:17)-HVR-L1-WYQQKPGKAPKLLIY (FR-L2, SEQ ID NO:18)-HVR-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, SEQ ID NO:19)-HVR-L3-FGQGTKVEIKR (FR-L4, SEQ ID NO:20). "Secretion signal sequence" or "signal sequence" refers to a nucleic acid sequence encoding a short signal peptide that can be used to direct a newly synthesized protein of interest through a cellular membrane, usually the inner membrane or both inner and outer membranes of prokaryotes. As such, the protein of interest such as the immunoglobulin light or heavy chain polypeptide is secreted into the periplasm of the prokaryotic host cells or into the culture medium. The signal peptide encoded by the secretion signal sequence may be endogenous to the host cells, or they may be exogenous, including signal peptides native to the polypeptide to be expressed. Secretion signal sequences are typically present at the amino terminus of a polypeptide to be expressed, and are typically removed enzymatically between biosynthesis and secretion of the polypeptide from the cytoplasm. Thus, the signal peptide is usually not present in a mature protein product.

"Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies

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generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (125I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen, et al., (1999) J. Mol. Biol. 293:865-881). To establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., (1997) Cancer Res. 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates have dried, 150 μl/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcoreTM-2000 or a BIAcoreTM-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams.

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The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881. If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stirred cuvette.

An "on-rate," "rate of association," "association rate," or "k_{on}" according to this invention can also be determined as described above using a BIAcore™-2000 or a BIAcore™-3000 system (BIAcore®, Inc., Piscataway, NJ).

A "disorder" is any condition or disease that would benefit from treatment with an substance/molecule or method of the invention. This includes chronic and acute disorders including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancerous conditions such as cancers or metastases of prostate, lung, and colon.

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

"Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal

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cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain , as well as head and neck cancer, and associated metastases.

A "STEAP-1-expressing cell" is a cell which expresses endogenous or transfected STEAP-1 on the cell surface. A "STEAP-1-expressing cancer" is a cancer comprising cells that have STEAP-1 protein present on the cell surface. A "STEAP-1-expressing cancer" produces sufficient levels of STEAP-1 on the surface of cells thereof, such that an anti-STEAP-1 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which "overexpresses" STEAP-1 is one which has significantly higher levels of STEAP-1 at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. STEAP-1 overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the STEAP-1 protein present on the surface of a cell (e.g. via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of STEAP-1-encoding nucleic acid or mRNA in the cell, e.g. via fluorescent in situ hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study STEAP-1 overexpression by measuring shed antigen in a biological fluid such as serum, e.g, using antibody-based assays (see also, e.g., U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias et al. J. Immunol. Methods 132: 73-80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A STEAP-1-expressing cancer includes prostate, lung, and colon cancer.

As used herein, "treatment" (and variations such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

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In some embodiments, antibodies of the invention are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). For prostate cancer, the progress of therapy can be assessed by routine methods, usually by measuring serum PSA (prostate specific antigen) levels; the higher the level of PSA in the blood, the more extensive the cancer. Commercial assays for detecting PSA are available, e.g, Hybitech Tandem-E and Tandem-R PSA assay kits, the Yang ProsCheck polyclonal assay (Yang Labs, Bellevue, WA), Abbott Imx (Abbott Labs, Abbott Park, IL), etc. Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver, respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

An "individual" is a vertebrate. In certain embodiments, the vertebrate is a mammal. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, a mammal is a human.

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a substance/molecule of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To

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the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Label" as used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-PSCA antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence which has been removed

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from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

"Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (*e.g.*, the ColE1 origin of replication) and a selectable marker (*e.g.*, ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic cells. Suitable vectors are disclosed below.

The cell that produces an anti-STEAP-1 antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a PSCA expressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of PSCA expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu), chemotherapeutic agents (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, toxins, growth inhibitory agents, drug moieties, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "toxin" is any substance capable of having a detrimental effect on the growth or proliferation of a cell.

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A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone;

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mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROCAL® etidronate, NE-58095, ZOMETA® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling

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pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell (such as a cell expressing STEAP-1) either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells (such as a cell expressing STEAP-1) in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "intracellular metabolite" refers to a compound resulting from a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC). The metabolic process or reaction may be an enzymatic process, such as proteolytic cleavage of a peptide linker of the ADC, or hydrolysis of a functional group such as a hydrazone, ester, or amide. Intracellular metabolites include, but are not limited to, antibodies and free drug which have undergone intracellular cleavage after entry, diffusion, uptake or transport into a cell.

The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC) whereby the covalent attachment, i.e. linker, between the drug moiety (D) and the antibody (Ab) is broken, resulting in the free drug dissociated from the antibody inside the cell. The cleaved moieties of the ADC are thus intracellular metabolites.

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The term "bioavailability" refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

The term "cytotoxic activity" refers to a cell-killing, cytostatic or growth inhibitory effect of an antibody-drug conjugate or an intracellular metabolite of an antibody-drug conjugate. Cytotoxic activity may be expressed as the IC_{50} value, which is the concentration (molar or mass) per unit volume at which half the cells survive.

"Alkyl" is C1-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH3), ethyl (Et, -CH2CH3), 1-propyl (n-Pr, n-propyl, -CH2CH2CH3), 2-propyl (i-Pr, i-propyl, -CH(CH3)2), 1-butyl (n-Bu, n-butyl, -CH2CH2CH2CH3), 2-methyl-1-propyl (i-Bu, i-butyl, -CH2CH(CH3)2), 2-butyl (s-Bu, s-butyl, -CH(CH3)CH2CH3), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH3)3), 1-pentyl (n-pentyl, -CH2CH2CH2CH2CH3), 2-pentyl (-CH(CH3)CH2CH2CH3), 3-pentyl (-CH(CH2CH3)2), 2-methyl-2-butyl (-C(CH3)2CH2CH3), 3-methyl-2-butyl (-CH(CH3)CH(CH3)2), 3-methyl-1-butyl (-CH2CH2CH(CH3)2), 2-methyl-1-butyl (-CH2CH(CH3)CH2CH3), 1-hexyl (-CH2CH2CH2CH2CH2CH3), 2-hexyl (-CH(CH3)CH2CH2CH2CH3), 3-hexyl (-CH(CH2CH3)(CH2CH2CH3)), 2-methyl-2-pentyl (-C(CH3)2CH2CH2CH3), 3-methyl-2-pentyl (-CH(CH3)CH(CH3)CH2CH3), 4-methyl-2-pentyl (-CH(CH3)CH2CH(CH3)2), 3-methyl-3-pentyl (-C(CH3)(CH2CH3)2), 2-methyl-3-pentyl (-CH(CH2CH3)CH(CH3)2), 2,3-dimethyl-2-butyl (-C(CH3)2CH(CH3)2), 3,3-dimethyl-2-butyl (-CH(CH3)C(CH3)3.

The term "C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl,-acetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isoheptyl, n-octyl, and

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isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -$N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Alkenyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH_2), allyl (-CH_2CH=CH_2), cyclopentenyl (-C_5H_7), and 5-hexenyl (-CH_2 $CH_2CH_2CH_2$CH=CH_2)

"Alkynyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic (-C≡CH) and propargyl (-CH_2C≡CH),

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH_2-) 1,2-ethyl (-CH_2CH_2-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

A "C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula -$(CH_2)_{1\text{-}10}$-. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

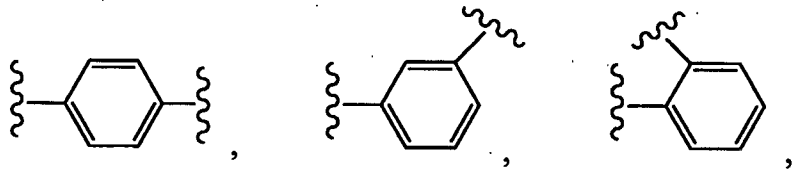
"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic

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group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

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"Substituted alkyl," "substituted aryl," and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, $-O^-$, -OR, -SR, $-S^-$, $-NR_2$, $-NR_3$, =NR, $-CX_3$, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, $-NO_2$, $=N_2$, $-N_3$, NC(=O)R, -C(=O)R, $-C(=O)NR_2$, $-SO_3^-$, $-SO_3H$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, -S(=O)R, $-OP(=O)(OR)_2$, $-P(=O)(OR)_2$, $-PO_3^-$, $-PO_3H_2$, -C(=O)R, -C(=O)X, -C(=S)R, $-CO_2R$, $-CO_2^-$, -C(=S)OR, -C(=O)SR, -C(=S)SR, $-C(=O)NR_2$, $-C(=S)NR_2$, $-C(=NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C_2-C_{18} alkyl, C_6-C_{20} aryl, C_3-C_{14} heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

"Heteroaryl" and "heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl,

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pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

A "C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -S(O)$_2$R', -S(O)R', -

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OH, -halogen, $-N_3$, $-NH_2$, -NH(R'), $-N(R')_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

A "C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', $-C(O)N(R')_2$ -NHC(O)R', $-S(O)_2R'$, -S(O)R', -OH, -halogen, $-N_3$, $-NH_2$, -NH(R'), $-N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

A "C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

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"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

"Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

Abbreviations

LINKER COMPONENTS:

MC = 6-maleimidocaproyl

Val-Cit or "vc" = valine-citrulline (an exemplary dipeptide in a protease cleavable linker)

Citrulline = 2-amino-5-ureido pentanoic acid

PAB = p-aminobenzyloxycarbonyl (an example of a "self immolative" linker component)

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Me-Val-Cit = N-methyl-valine-citrulline (wherein the linker peptide bond has been modified to prevent its cleavage by cathepsin B)

MC(PEG)6-OH = maleimidocaproyl- polyethylene glycol (can be attached to antibody cysteines).

SPP = N-succinimidyl-4-(2-pyridylthio)pentanoate

SPDP = N-succinimidyl-3-(2-pyridyldithio) propionate

SMCC = succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate

IT = iminothiolane

CYTOTOXIC DRUGS:

MMAE = mono-methyl auristatin E (MW 718)

MMAF = variant of auristatin E (MMAE) with a phenylalanine at the C-terminus of the drug (MW 731.5)

MMAF-DMAEA = MMAF with DMAEA (dimethylaminoethylamine) in an amide linkage to the C-terminal phenylalanine (MW 801.5)

MMAF-TEG = MMAF with tetraethylene glycol esterified to the phenylalanine

MMAF-NtBu = N-t-butyl, attached as an amide to C-terminus of MMAF

DM1 = N(2')-deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine

DM3 = N(2')-deacetyl-N2-(4-mercapto-1-oxopentyl)-maytansine

DM4 = N(2')-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl)-maytansine

Further abbreviations are as follows: AE is auristatin E, Boc is N-(t-butoxycarbonyl), cit is citrulline, dap is dolaproine, DCC is 1,3-dicyclohexylcarbodiimide, DCM is dichloromethane, DEA is diethylamine, DEAD is diethylazodicarboxylate, DEPC is diethylphosphorylcyanidate, DIAD is diisopropylazodicarboxylate, DIEA is N,N-diisopropylethylamine, dil is dolaisoleucine, DMA is dimethylacetamide, DMAP is 4-dimethylaminopyridine, DME is ethyleneglycol dimethyl ether (or 1,2-dimethoxyethane), DMF is N,N-dimethylformamide, DMSO is dimethylsulfoxide, doe is dolaphenine, dov is N,N-dimethylvaline, DTNB is 5,5'-dithiobis(2-nitrobenzoic acid), DTPA is diethylenetriaminepentaacetic acid, DTT is dithiothreitol, EDCI is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EEDQ is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ES-MS is electrospray mass spectrometry, EtOAc is ethyl acetate, Fmoc is N-(9-fluorenylmethoxycarbonyl), gly is glycine, HATU is O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, HOBt is 1-hydroxybenzotriazole, HPLC is high pressure liquid chromatography, ile is isoleucine, lys is lysine,

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MeCN (CH_3CN) is acetonitrile, MeOH is methanol, Mtr is 4-anisyldiphenylmethyl (or 4-methoxytrityl),nor is (1S, 2R)-(+)-norephedrine, PBS is phosphate-buffered saline (pH 7.4), PEG is polyethylene glycol, Ph is phenyl, Pnp is p-nitrophenyl, MC is 6-maleimidocaproyl, phe is L-phenylalanine, PyBrop is bromo tris-pyrrolidino phosphonium hexafluorophosphate, SEC is size-exclusion chromatography, Su is succinimide, TFA is trifluoroacetic acid, TLC is thin layer chromatography, UV is ultraviolet, and val is valine.

COMPOSITIONS AND METHODS OF MAKING THE SAME

Antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as cancer.

Anti-STEAP-1 Antibodies

In one aspect, the invention provides antibodies that bind to STEAP-1. In some embodiments, antibodies are provided that bind to a mature form of human and cynomolgus monkey (cyno) STEAP-1. In one such embodiment, a mature form of human STEAP-1 has an amino acid sequence of SEQ ID NO:1 (Figure 1). The cyno STEAP-1 has an amino acid sequence of SEQ ID NO:3 (Figure 1). In some embodiments, an antibody to STEAP-1 binds to a mature form of STEAP-1 expressed on the cell surface. In some embodiments, an antibody that binds to a mature form of STEAP-1 expressed on the cell surface inhibits the growth of the cell. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and inhibits cell proliferation. In certain embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and induces cell death. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the surface of cancer cells. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 that is overexpressed on the surface of cancer cells relative to normal cells of the same tissue origin. In some embodiments, an anti-STEAP-1 antibody is conjugated to a cytotoxin or a detectable label and binds to STEAP-1 on a cell surface. In some embodiments, the antibody-toxin conjugate inhibits growth of the cell. In some embodiments, the antibody-detectable label conjugate causes a cell expressing STEAP-1 on its surface to be detectable in vitro or in vivo.

In one aspect, an anti-STEAP-1 antibody is a monoclonal antibody. In one aspect, an anti-STEAP-1 antibody is an antibody fragment, e.g., a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an

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anti-STEAP-1 antibody is a chimeric, humanized, or human antibody. In one aspect, any of the anti-STEAP-1 antibodies described herein are purified.

Exemplary monoclonal antibodies derived from a phage library are provided herein. The antigen used for screening the library was a polypeptide having the sequence of amino acid sequences of SEQ ID NO:28 or SEQ ID NO:30, corresponding to the extracellular domains (ECDs) of STEAP-1 beta and alpha. The antibodies resulting from the library screen are affinity matured.

In one aspect, monoclonal antibodies that compete with murine 120.545, 120 graft, and humanized 120v.24 binding to STEAP-1 are provided. Monoclonal antibodies that bind to the same epitope as murine 120.545, 120 graft, and humanized 120v.24 are also provided.

In one aspect of the invention, polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the invention, compositions comprising anti-STEAP-1 antibodies or polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, a composition of the invention is a pharmaceutical formulation for the treatment of a cell proliferative disorder, such as those enumerated herein.

A detailed description of exemplary anti-STEAP-1 antibodies is as follows:

1. Specific embodiments of anti-STEAP-1 antibodies

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain comprising SEQ ID NO:9 or 10 of Figure 2B. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising SEQ ID NO:6 of Figure 2A.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain comprising SEQ ID NO:9, having one or more of the following amino changes at the indicated Kabat position: A24V, V37I, V48M, F67I, and L78F. In one embodiment, the heavy chain comprises a heavy chain framework region selected from SEQ ID NOs:25, 75, 76, 77, 78, and 79. As used herein, heavy chain framework regions are designated "FR-H1-H4" or "HC-FR1-FR4," and light chain framework regions are designated "FR-L1-L4" or "LC-FR1-FR4." In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain comprising SEQ ID NO:6.

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In one aspect, the invention provides an anti-STEAP-1 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the antibody 120.v24 shown in Figures 2A and 2B.

An anti-STEAP-1 antibody may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind STEAP-1. For example, in some embodiments, anti-STEAP-1 antibodies of the invention comprise a human subgroup III heavy chain framework consensus sequence. In one embodiment of these antibodies, the heavy chain framework consensus sequence comprises substitution(s) at position 24, 37, 48, 67, and/or 78. In one embodiment of these antibodies, position 24 is A or V, position 37 is I or V, position 48 is M or V, position 67 is I or F, and/or position 78 is F or L. In one embodiment, these antibodies comprise a heavy chain variable domain framework sequence of huMAb4D5-8, e.g., SEQ ID NO:21, 22, 23, and 24 (FR-H1, FR-H2, FR-H3, FR-H4, respectively). huMAb4D5-8 is commercially known as HERCEPTIN® anti-HER2 antibody, Genentech, Inc., South San Francisco, CA, USA; also referred to in U.S. Pat. Nos. 6,407,213 & 5,821,337, and Lee et al., J. Mol. Biol. (2004), 340(5):1073-93. In one such embodiment, these antibodies further comprise a human κI light chain framework consensus sequence. In one such embodiment, these antibodies comprise a light chain variable domain framework sequence of huMAb4D5-8, e.g. SEQ ID NO:17, 18, 19, and 20 (FR-L1, FR-L2, FR-L3, FR-L4, respectively).

In one embodiment, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the FR-H1-FR-H4 sequences SEQ ID NO:21 or 25 (FR-H1), 22 (FR-H2), 23 (FR-H3), and 24 (FR-H4), respectively; the HVR H1 comprises the amino acid sequence of SEQ ID NO:14; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:15; and the HVR-H3 comprises an amino acid sequence of SEQ ID NO:16. In one embodiment, an anti-STEAP-1 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the FR-L1-FR-L4 sequences of SEQ ID NOs:17, 18, 19 and 20, respectively; the HVR-L1 comprises the amino acid sequence selected from SEQ ID NOs:11, 12, and 13. In one embodiment of these antibodies, the heavy chain variable domain comprises SEQ ID NOs:9 or 10 and the light chain variable domain comprises SEQ ID NO:6.

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:9 or 10. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence,

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but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence SEQ ID NOs:9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, and/or 79. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NO:9 or 10.

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain as depicted in in Figure 2B (SEQ ID NOs:9 or 10).

In some embodiments, the heavy chain HVR and FR sequences comprise the following:

HVR-H1 (GYSITSDYAWN, SEQ ID NO:14)

HVR-H2 (GYISNSGSTSYNPSLKS, SEQ ID NO:15)

HVR-H3 (ERNYDYDDYYYAMDY, SEQ ID NO:16)

FR-H1 (EVQLVESGGGLVQPGGSLRLSCAAS, SEQ ID NO:21)

FR-H1 (EVQLVESGGGLVQPGGSLRLSCAVS, SEQ ID NO:25)

FR-H2 (WVRQAPGKGLEWV, SEQ ID NO:22)

FR-H2 (WIRQAPGKGLEWV, SEQ ID NO:75)

FR-H2 (WVRQAPGKGLEWM, SEQ ID NO:76)

FR-H2 (WIRQAPGKGLEWM, SEQ ID NO:77)

FR-H3 (RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR, SEQ ID NO:23)

FR-H3 (RITISRDNSKNTLYLQMNSLRAEDTAVYYCAR, SEQ ID NO:78)

FR-H3 (RFTISRDNSKNTFYLQMNSLRAEDTAVYYCAR, SEQ ID NO:79)

FR-H4 (WGQGTLVTVSS, SEQ ID NO:24)

In some embodiments, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain as depicted in Figure 2A (SEQ ID NO:6).

In some embodiments, the light chain HVR sequences comprise the following:

HVR-L1(KSSQSLLYRSNQKNYLA, SEQ ID NO:11)

HVR-L2 (WASTRES, SEQ ID NO:12)

HVR-L3 (QQYYNYPRT, SEQ ID NO:13).

In some embodiments, the light chain FR sequences comprise the following:

FR-L1 (DIQMTQSPSSLSASVGDRVTITC, SEQ ID NO:17);

FR-L2 (WYQQKPGKAPKLLIY, SEQ ID NO:18);

FR-L3 (GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC, SEQ ID NO:19)

FR-L4 (FGQGTKVEIKR, SEQ ID NO:20).

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In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from SEQ ID NOs:6, 11, 12, 13, 17, 18, 19, and 20. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a light chain variable domain comprising the amino acid sequence SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NO:9 and 10; and (b) a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in the reference sequence, including but not limited to a sequence selected from SEQ ID NOs:9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, 79. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:9 or 10 and a light chain variable domain comprising an amino acid sequence selected from SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Figure 2B and/or (b) one, two, or three VL HVRs selected from those shown in Figure 2A. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain selected from those shown in Figure 2B and a light chain variable domain selected from those shown in Figure 2A.

2. Antibody Fragments

The present invention encompasses antibody fragments. Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances

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there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

3. Humanized Antibodies

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

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antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

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

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

4. Human Antibodies

Human anti-STEAP-1 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s) as described above. Alternatively, human monoclonal anti-STEAP-1 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).

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It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255 (1993); Bruggermann et al., Year in Immunol., 7: 33 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

5. Bispecific Antibodies

Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for STEAP-1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of STEAP-1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express STEAP-1. These antibodies possess a STEAP-1-binding arm and an arm which binds a cytotoxic agent, such as, e.g., saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light

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chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305: 537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker et al., EMBO J., 10: 3655 (1991).

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

In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large

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amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

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

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA,

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90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

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

6. Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. In certain embodiments, the dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. In certain embodiments, a multivalent antibody comprises (or consists of) three to about eight antigen binding sites. In one such embodiment, a multivalent antibody comprises (or consists of) four antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (for example, two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n -VD2-(X2)n -Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein may further comprise at least two (for example, four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

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7. Single-Domain Antibodies

In some embodiments, an antibody of the invention is a single-domain antibody. A single-domain antibody is a single polyeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

8. Antibody Variants

In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

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

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody

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with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

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

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

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and US Patent No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient"

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antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)).

In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US 5739277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (US 2003/0190311, US6821505; US 6165745; US 5624821; US 5648260; US 6165745;US 5834 597).

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

TABLE 1

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp

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Original Residue	Exemplary Substitutions	Preferred Substitutions
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

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(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

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

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

It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a

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human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions including that of a hinge cysteine.

In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the invention may comprise one or more alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001). Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

In one aspect, the invention provides antibodies comprising modifications in the interface of Fc polypeptides comprising the Fc region, wherein the modifications facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance is positionable in the cavity so as to promote complexing of the first and second Fc polypeptides. Methods of generating antibodies with these modifications are known in the art, e.g., as described in U.S. Pat. No. 5,731,168.

9. Antibody Derivatives

The antibodies of the present invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene

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glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

Certain Methods of Making Antibodies

1. Certain Hybridoma-Based Methods

The anti-STEAP-1 monoclonal antibodies of the invention can be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies to STEAP-1 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of STEAP-1 and an adjuvant. STEAP-1 may be prepared using methods well-known in the art, some of which are further described herein. For example, STEAP-1 may be produced recombinantly. In one embodiment, animals are immunized with a derivative of STEAP-1 that contains an extracellular portion of STEAP-1 fused to the Fc portion of an immunoglobulin heavy chain. In one embodiment, animals are immunized with an STEAP-1-IgG1 fusion protein. In one embodiment, animals are immunized with immunogenic derivatives of STEAP-1 in a

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solution with monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT), and the solution is injected intradermally at multiple sites. Two weeks later the animals are boosted. Seven to fourteen days later the animals are bled, and the serum is assayed for anti- STEAP-1 titer. Animals are boosted until titer plateaus.

Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

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

In certain embodiments, myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies that bind to STEAP-1. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

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Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

2. Certain Library Screening Methods

Anti-STEAP-1 antibodies of the invention can be made by using combinatorial libraries to screen for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are described generally in Hoogenboom et al. (2001) in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ), and in certain embodiments, in Lee et al. (2004) J. Mol. Biol. 340:1073-1093.

In principle, synthetic antibody clones are selected by screening phage libraries containing phage that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are panned by affinity chromatography against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further enriched by additional cycles of antigen adsorption/elution. Any of the anti-STEAP-1 antibodies of the invention can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-STEAP-1 antibody clone using the Fv sequences from the phage clone of interest and suitable constant region (Fc) sequences described in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

In certain embodiments, the antigen-binding domain of an antibody is formed from two variable (V) regions of about 110 amino acids, one each from the light (VL) and heavy (VH) chains, that both present three hypervariable loops (HVRs) or complementarity-determining regions (CDRs). Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). As used herein, scFv encoding phage clones and Fab encoding phage clones are collectively referred to as "Fv phage clones" or "Fv clones."

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Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

In certain embodiments, filamentous phage is used to display antibody fragments by fusion to the minor coat protein pIII. The antibody fragments can be displayed as single chain Fv fragments, in which VH and VL domains are connected on the same polypeptide chain by a flexible polypeptide spacer, e.g. as described by Marks et al., J. Mol. Biol., 222: 581-597 (1991), or as Fab fragments, in which one chain is fused to pIII and the other is secreted into the bacterial host cell periplasm where assembly of a Fab-coat protein structure which becomes displayed on the phage surface by displacing some of the wild type coat proteins, e.g. as described in Hoogenboom et al., Nucl. Acids Res., 19: 4133-4137 (1991).

In general, nucleic acids encoding antibody gene fragments are obtained from immune cells harvested from humans or animals. If a library biased in favor of anti-STEAP-1 clones is desired, the subject is immunized with STEAP-1 to generate an antibody response, and spleen cells and/or circulating B cells other peripheral blood lymphocytes (PBLs) are recovered for library construction. In a preferred embodiment, a human antibody gene fragment library biased in favor of anti-STEAP-1 clones is obtained by generating an anti-STEAP-1 antibody response in transgenic mice carrying a functional human immunoglobulin gene array (and lacking a functional endogenous antibody production system) such that STEAP-1 immunization gives rise to B cells producing human antibodies against STEAP-1. The generation of human antibody-producing transgenic mice is described below.

Additional enrichment for anti-STEAP-1 reactive cell populations can be obtained by using a suitable screening procedure to isolate B cells expressing STEAP-1-specific membrane bound antibody, e.g., by cell separation using STEAP-1 affinity chromatography or adsorption of cells to fluorochrome-labeled STEAP-1 followed by flow-activated cell sorting (FACS).

Alternatively, the use of spleen cells and/or B cells or other PBLs from an unimmunized donor provides a better representation of the possible antibody repertoire, and also permits the construction of an

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antibody library using any animal (human or non-human) species in which STEAP-1 is not antigenic. For libraries incorporating in vitro antibody gene construction, stem cells are harvested from the subject to provide nucleic acids encoding unrearranged antibody gene segments. The immune cells of interest can be obtained from a variety of animal species, such as human, mouse, rat, lagomorpha, luprine, canine, feline, porcine, bovine, equine, and avian species, etc.

Nucleic acid encoding antibody variable gene segments (including VH and VL segments) are recovered from the cells of interest and amplified. In the case of rearranged VH and VL gene libraries, the desired DNA can be obtained by isolating genomic DNA or mRNA from lymphocytes followed by polymerase chain reaction (PCR) with primers matching the 5' and 3' ends of rearranged VH and VL genes as described in Orlandi et al., Proc. Natl. Acad. Sci. (USA), 86: 3833-3837 (1989), thereby making diverse V gene repertoires for expression. The V genes can be amplified from cDNA and genomic DNA, with back primers at the 5' end of the exon encoding the mature V-domain and forward primers based within the J-segment as described in Orlandi et al. (1989) and in Ward et al., Nature, 341: 544-546 (1989). However, for amplifying from cDNA, back primers can also be based in the leader exon as described in Jones et al., Biotechnol., 9: 88-89 (1991), and forward primers within the constant region as described in Sastry et al., Proc. Natl. Acad. Sci. (USA), 86: 5728-5732 (1989). To maximize complementarity, degeneracy can be incorporated in the primers as described in Orlandi et al. (1989) or Sastry et al. (1989). In certain embodiments, library diversity is maximized by using PCR primers targeted to each V-gene family in order to amplify all available VH and VL arrangements present in the immune cell nucleic acid sample, e.g. as described in the method of Marks et al., J. Mol. Biol., 222: 581-597 (1991) or as described in the method of Orum et al., Nucleic Acids Res., 21: 4491-4498 (1993). For cloning of the amplified DNA into expression vectors, rare restriction sites can be introduced within the PCR primer as a tag at one end as described in Orlandi et al. (1989), or by further PCR amplification with a tagged primer as described in Clackson et al., Nature, 352: 624-628 (1991).

Repertoires of synthetically rearranged V genes can be derived in vitro from V gene segments. Most of the human VH-gene segments have been cloned and sequenced (reported in Tomlinson et al., J. Mol. Biol., 227: 776-798 (1992)), and mapped (reported in Matsuda et al., Nature Genet., 3: 88-94 (1993); these cloned segments (including all the major conformations of the H1 and H2 loop) can be used to generate diverse VH gene repertoires with PCR primers encoding H3 loops of diverse sequence and length as described in Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). VH repertoires can also be made with all the sequence diversity focused in a long H3 loop of a single length as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 89: 4457-4461 (1992). Human Vκ and Vλ segments have been cloned and sequenced (reported in Williams and Winter, Eur. J. Immunol., 23: 1456-1461 (1993)) and can be used to

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make synthetic light chain repertoires. Synthetic V gene repertoires, based on a range of VH and VL folds, and L3 and H3 lengths, will encode antibodies of considerable structural diversity. Following amplification of V-gene encoding DNAs, germline V-gene segments can be rearranged in vitro according to the methods of Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

Repertoires of antibody fragments can be constructed by combining VH and VL gene repertoires together in several ways. Each repertoire can be created in different vectors, and the vectors recombined in vitro, e.g., as described in Hogrefe et al., Gene, 128: 119-126 (1993), or in vivo by combinatorial infection, e.g., the loxP system described in Waterhouse et al., Nucl. Acids Res., 21: 2265-2266 (1993). The in vivo recombination approach exploits the two-chain nature of Fab fragments to overcome the limit on library size imposed by E. coli transformation efficiency. Naive VH and VL repertoires are cloned separately, one into a phagemid and the other into a phage vector. The two libraries are then combined by phage infection of phagemid-containing bacteria so that each cell contains a different combination and the library size is limited only by the number of cells present (about 10^{12} clones). Both vectors contain in vivo recombination signals so that the VH and VL genes are recombined onto a single replicon and are co-packaged into phage virions. These huge libraries provide large numbers of diverse antibodies of good affinity (K_d^{-1} of about 10^{-8} M).

Alternatively, the repertoires may be cloned sequentially into the same vector, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 88: 7978-7982 (1991), or assembled together by PCR and then cloned, e.g. as described in Clackson et al., Nature, 352: 624-628 (1991). PCR assembly can also be used to join VH and VL DNAs with DNA encoding a flexible peptide spacer to form single chain Fv (scFv) repertoires. In yet another technique, "in cell PCR assembly" is used to combine VH and VL genes within lymphocytes by PCR and then clone repertoires of linked genes as described in Embleton et al., Nucl. Acids Res., 20: 3831-3837 (1992).

The antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_d^{-1} of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked in vitro by constructing and reselecting from secondary libraries as described in Winter et al. (1994), supra. For example, mutation can be introduced at random in vitro by using error-prone polymerase (reported in Leung et al., Technique, 1: 11-15 (1989)) in the method of Hawkins et al., J. Mol. Biol., 226: 889-896 (1992) or in the method of Gram et al., Proc. Natl. Acad. Sci USA, 89: 3576-3580 (1992). Additionally, affinity maturation can be performed by randomly mutating one or more CDRs, e.g. using PCR with primers carrying random sequence spanning the CDR of interest, in selected individual Fv clones and screening for higher affinity clones. WO 9607754 (published 14 March 1996) described a method for inducing mutagenesis in a

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complementarity determining region of an immunoglobulin light chain to create a library of light chain genes. Another effective approach is to recombine the VH or VL domains selected by phage display with repertoires of naturally occurring V domain variants obtained from unimmunized donors and screen for higher affinity in several rounds of chain reshuffling as described in Marks et al., Biotechnol., 10: 779-783 (1992). This technique allows the production of antibodies and antibody fragments with affinities of about 10^{-9} M or less.

Screening of the libraries can be accomplished by various techniques known in the art. For example, STEAP-1 can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning phage display libraries.

The phage library samples are contacted with immobilized STEAP-1 under conditions suitable for binding at least a portion of the phage particles with the adsorbent. Normally, the conditions, including pH, ionic strength, temperature and the like are selected to mimic physiological conditions. The phages bound to the solid phase are washed and then eluted by acid, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci USA, 88: 7978-7982 (1991), or by alkali, e.g. as described in Marks et al., J. Mol. Biol., 222: 581-597 (1991), or by STEAP-1 antigen competition, e.g. in a procedure similar to the antigen competition method of Clackson et al., Nature, 352: 624-628 (1991). Phages can be enriched 20-1,000-fold in a single round of selection. Moreover, the enriched phages can be grown in bacterial culture and subjected to further rounds of selection.

The efficiency of selection depends on many factors, including the kinetics of dissociation during washing, and whether multiple antibody fragments on a single phage can simultaneously engage with antigen. Antibodies with fast dissociation kinetics (and weak binding affinities) can be retained by use of short washes, multivalent phage display and high coating density of antigen in solid phase. The high density not only stabilizes the phage through multivalent interactions, but favors rebinding of phage that has dissociated. The selection of antibodies with slow dissociation kinetics (and good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass et al., Proteins, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks et al., Biotechnol., 10: 779-783 (1992).

It is possible to select between phage antibodies of different affinities, even with affinities that differ slightly, for STEAP-1. However, random mutation of a selected antibody (e.g. as performed in some affinity maturation techniques) is likely to give rise to many mutants, most binding to antigen, and a few with higher affinity. With limiting STEAP-1, rare high affinity phage could be competed out. To retain all

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higher affinity mutants, phages can be incubated with excess biotinylated STEAP-1, but with the biotinylated STEAP-1 at a concentration of lower molarity than the target molar affinity constant for STEAP-1. The high affinity-binding phages can then be captured by streptavidin-coated paramagnetic beads. Such "equilibrium capture" allows the antibodies to be selected according to their affinities of binding, with sensitivity that permits isolation of mutant clones with as little as two-fold higher affinity from a great excess of phages with lower affinity. Conditions used in washing phages bound to a solid phase can also be manipulated to discriminate on the basis of dissociation kinetics.

Anti-STEAP-1 clones may be selected based on activity. In certain embodiments, the invention provides anti-STEAP-1 antibodies that bind to living cells that naturally express STEAP-1. In one embodiment, the invention provides anti-STEAP-1 antibodies that block the binding between a STEAP-1 ligand and STEAP-1, but do not block the binding between a STEAP-1 ligand and a second protein. Fv clones corresponding to such anti-STEAP-1 antibodies can be selected by (1) isolating anti-STEAP-1 clones from a phage library as described above, and optionally amplifying the isolated population of phage clones by growing up the population in a suitable bacterial host; (2) selecting STEAP-1 and a second protein against which blocking and non-blocking activity, respectively, is desired; (3) adsorbing the anti-STEAP-1 phage clones to immobilized STEAP-1; (4) using an excess of the second protein to elute any undesired clones that recognize STEAP-1-binding determinants which overlap or are shared with the binding determinants of the second protein; and (5) eluting the clones which remain adsorbed following step (4). Optionally, clones with the desired blocking/non-blocking properties can be further enriched by repeating the selection procedures described herein one or more times.

DNA encoding hybridoma-derived monoclonal antibodies or phage display Fv clones of the invention is readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide primers designed to specifically amplify the heavy and light chain coding regions of interest from hybridoma or phage DNA template). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of the desired monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs, 130: 151 (1992).

DNA encoding the Fv clones of the invention can be combined with known DNA sequences encoding heavy chain and/or light chain constant regions (e.g. the appropriate DNA sequences can be obtained from Kabat et al., supra) to form clones encoding full or partial length heavy and/or light chains.

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It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. An Fv clone derived from the variable domain DNA of one animal (such as human) species and then fused to constant region DNA of another animal species to form coding sequence(s) for "hybrid," full length heavy chain and/or light chain is included in the definition of "chimeric" and "hybrid" antibody as used herein. In certain embodiments, an Fv clone derived from human variable DNA is fused to human constant region DNA to form coding sequence(s) for full- or partial-length human heavy and/or light chains.

DNA encoding anti-STEAP-1 antibody derived from a hybridoma of the invention can also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of homologous murine sequences derived from the hybridoma clone (e.g. as in the method of Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). DNA encoding a hybridoma- or Fv clone-derived antibody or fragment can be further modified by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In this manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the Fv clone or hybridoma clone-derived antibodies of the invention.

3. Vectors, Host Cells, and Recombinant Methods

For recombinant production of an antibody of the invention, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating antibodies using prokaryotic host cells:

Vector Construction

Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can

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be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically transformed using pBR322, a plasmid derived from an E. coli species. pBR322 contains genes encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Patent No. 5,648,237.

In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λGEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as E. coli LE392.

The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter

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sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β-galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

In another aspect, the production of the immunoglobulins according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB- strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

Antibodies of the invention can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

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One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a "codon bank" at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) METHODS: A Companion to Methods in Enzymol. 4:151-158.

In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5, 840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the invention.

Prokaryotic host cells suitable for expressing antibodies of the invention include Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., E. coli), Bacilli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the invention. Examples of E. coli strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli B, E. coliλ 1776 (ATCC 31,537) and E. coli RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is

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generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

Antibody Production

Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

Prokaryotic cells used to produce the polypeptides of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for E. coli growth, growth temperatures range from about 20°C to about 39°C; from about 25°C to about 37°C; or about 30°C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly

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on the host organism. In certain embodiments, for E. coli, the pH is from about 6.8 to about 7.4, or about 7.0.

If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al., J. Immunol. Methods (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

In one embodiment, the expressed polypeptides of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

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To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Patent No. 6,083,715; Georgiou et al., U.S. Patent No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Patent No. 5,264,365; Georgiou et al., U.S. Patent No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

In one embodiment, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

Antibody Purification

In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the invention. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica

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surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating antibodies using eukaryotic host cells:

A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

Signal sequence component

A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

Origin of replication

Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

Selection gene component

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and

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thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

Promoter component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (e.g., an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

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The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982), describing expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

Enhancer element component

Transcription of DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is generally located at a site 5' from the promoter.

Transcription termination component

Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

Selection and transformation of host cells

Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or

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293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)) ; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Culturing the host cells

The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Purification of antibody

When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration.

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Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Immunoconjugates

The invention also provides immunoconjugates (interchangeably referred to as "antibody-drug conjugates," or "ADCs") comprising any of the anti-STEAP-1 antibodies of the invention conjugated to one or more cytotoxic agents, such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin

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(e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and a chemotherapeutic agent or other toxin. Chemotherapeutic agents useful in the generation of immunoconjugates are described herein (e.g., above). Enzymatically active toxins and fragments thereof can also be used and are described herein.

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and one or more small molecule toxins, including, but not limited to, small molecule drugs such as a calicheamicin, maytansinoid, dolastatin, auristatin, trichothecene, and CC1065, and the derivatives of these drugs that have cytotoxic activity. Examples of such immunoconjugates are discussed in further detail below.

1. Exemplary Immunoconjugates

An immunoconjugate (or "antibody-drug conjugate" ("ADC")) of the invention may be of Formula I, below, wherein an anti-STEAP-1 antibody is conjugated (i.e., covalently attached) to one or more drug moieties (D) through an optional linker (L).

$$Ab–(L–D)_p \qquad \text{Formula I}$$

Accordingly, the anti-STEAP-1 antibody may be conjugated to the drug either directly or via a linker. In Formula I, p is the average number of drug moieties per antibody, which can range, e.g., from about 1 to about 20 drug moieties per antibody, and in certain embodiments, from 1 to about 8 drug moieties per antibody.

Exemplary Linkers

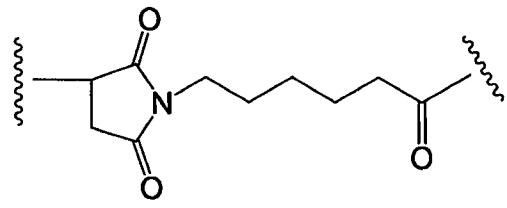
Exemplary linkers and drug moieties are disclosed herein. A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). Various linker components are known in the art, some of which are described below.

A linker may be a "cleavable linker," facilitating release of a drug in the cell. For example, an acid-labile linker (e.g., hydrazone), protease-sensitive (e.g., peptidase-sensitive) linker, photolabile linker,

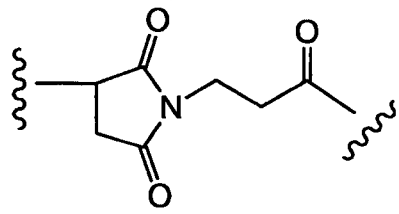
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dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

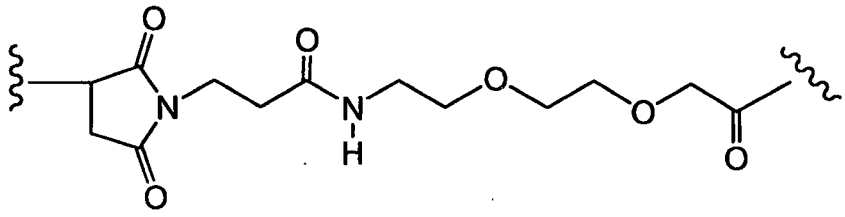
In some embodiments, a linker component may comprise a "stretcher unit" that links an antibody to another linker component or to a drug moiety. Exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody):



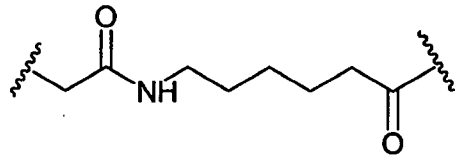
MC



MP



MPEG



In some embodiments, a linker component may comprise an amino acid unit. In one such embodiment, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes. See, e.g., Doronina et al. (2003) Nat. Biotechnol. 21:778-784. Exemplary amino acid units include, but are not limited to, a dipeptide, a tripeptide, a tetrapeptide, and a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); or N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include: glycine-

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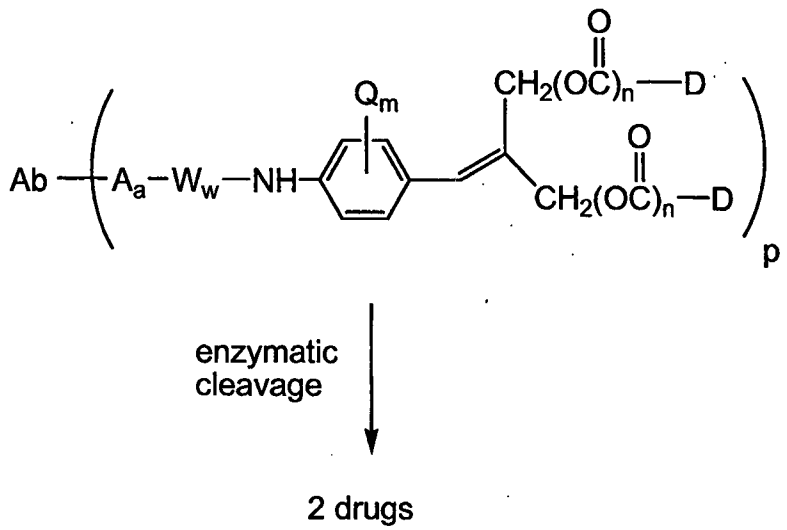
valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

In some embodiments, a linker component may comprise a "spacer" unit that links the antibody to a drug moiety, either directly or by way of a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon enzymatic (e.g., proteolytic) cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. Other combinations of peptidic spacers susceptible to sequence-specific enzymatic cleavage are also contemplated. For example, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease would result in release of a glycine-glycine-drug moiety from the remainder of the ADC. In one such embodiment, the glycine-glycine-drug moiety is then subjected to a separate hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

A "self-immolative" spacer unit allows for release of the drug moiety without a separate hydrolysis step. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In one such embodiment, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and a cytotoxic agent. See, e.g., Hamann et al. (2005) Expert Opin. Ther. Patents (2005) 15:1087-1103. In one embodiment, the spacer unit is p-aminobenzyloxycarbonyl (PAB). In certain embodiments, the phenylene portion of a p-amino benzyl unit is substituted with Qm, wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen,- nitro or -cyano; and m is an integer ranging from 0-4. Examples of self-immolative spacer units further include, but are not limited to, aromatic compounds that are electronically similar to p-aminobenzyl alcohol (see, e.g., US 2005/0256030 A1), such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho- or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., Chemistry Biology, 1995, 2, 223); appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, et al., J. Amer. Chem. Soc., 1972, 94, 5815); and 2-aminophenylpropionic acid amides (Amsberry, et al., J. Org. Chem., 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the a-position of glycine (Kingsbury, et al., J. Med. Chem., 1984, 27, 1447) are also examples of self-immolative spacers useful in ADCs.

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In one embodiment, a spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted below, which can be used to incorporate and release multiple drugs.



wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

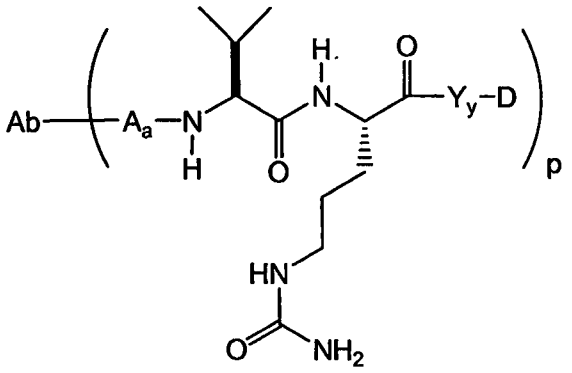
A linker may comprise any one or more of the above linker components. In certain embodiments, a linker is as shown in brackets in the following ADC Formula II

$Ab-([Aa-Ww-Yy]-D)_p$ Formula II

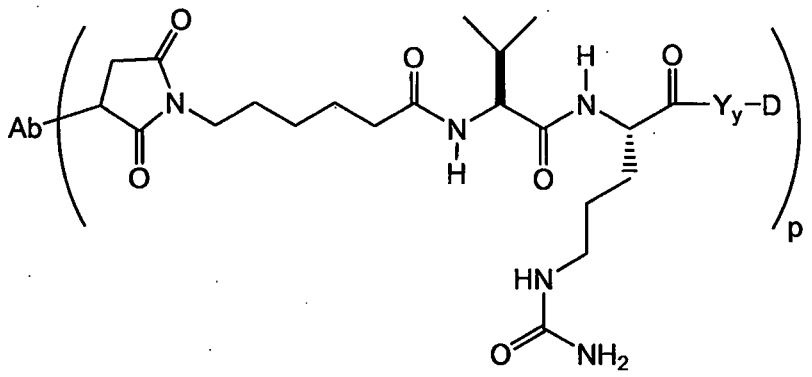
wherein A is a stretcher unit, and a is an integer from 0 to 1; W is an amino acid unit, and w is an integer from 0 to 12; Y is a spacer unit, and y is 0, 1, or 2; and Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in US 20050238649 A1, which is expressly incorporated herein by reference.

Exemplary linker components and combinations thereof are shown below in the context of ADCs of Formula II:

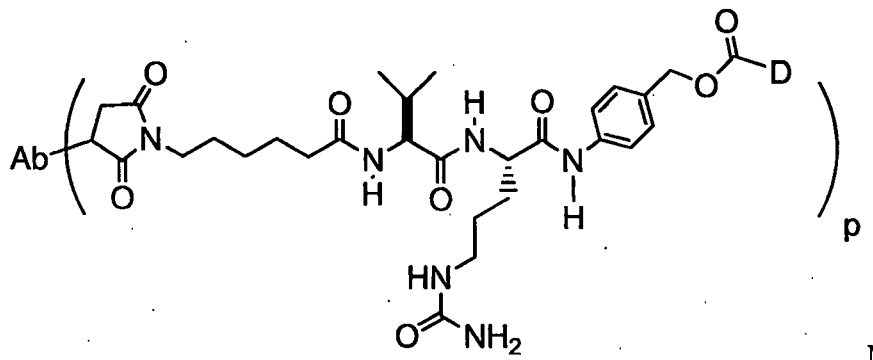
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Val-Cit or VC



MC-val-cit



MC-val-cit-PAB

Linkers components, including stretcher, spacer, and amino acid units, may be synthesized by methods known in the art, such as those described in US 2005-0238649 A1.

Exemplary Drug Moieties

Maytansine and maytansinoids

In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to one or more maytansinoid molecules. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids,

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such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

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 or produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973). Maytansinol and maytansinol analogues may also be prepared synthetically according to known methods.

Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, as disclosed herein.

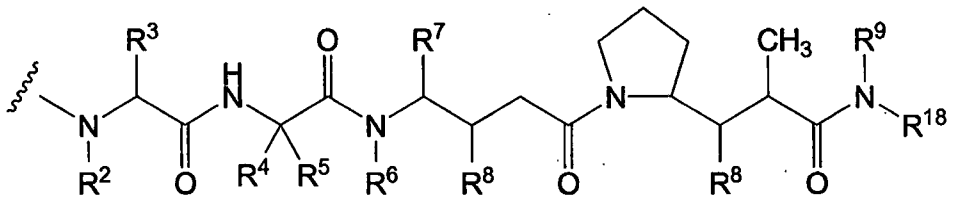
Auristatins and dolastatins

In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to dolastatin or a dolastatin peptidic analog or derivative, e.g., an auristatin (US Pat. Nos. 5635483; 5780588). 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 (US Pat. No.5663149) 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).

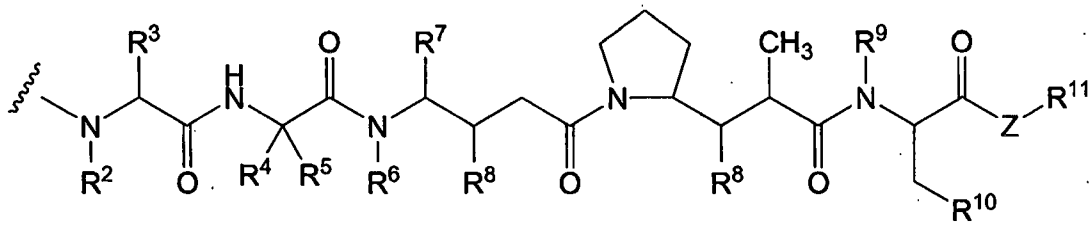
Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

A peptidic drug moiety may be selected from Formulas D_E and D_F below:

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D_E



D_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O-(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

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each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n$-COOH;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$–aryl, $-C(R^8)_2-C(R^8)_2-(C_3$-C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3$-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment, R^3, R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is –H or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is -H, and R^7 is sec-butyl.

In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is -H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is -H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is -H.

In one embodiment, Z is -O- or -NH-.

In one embodiment, R^{10} is aryl.

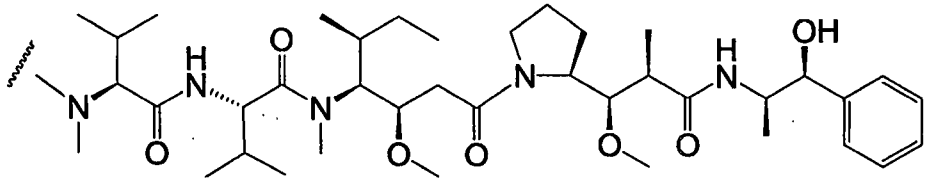
In an exemplary embodiment, R^{10} is -phenyl.

In an exemplary embodiment, when Z is -O-, R^{11} is –H, methyl or t-butyl.

In one embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is -C_1-C_8 alkyl or $-(CH_2)_n$-COOH.

In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

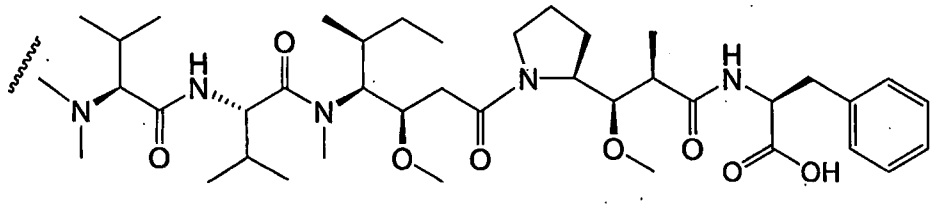
An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



MMAE

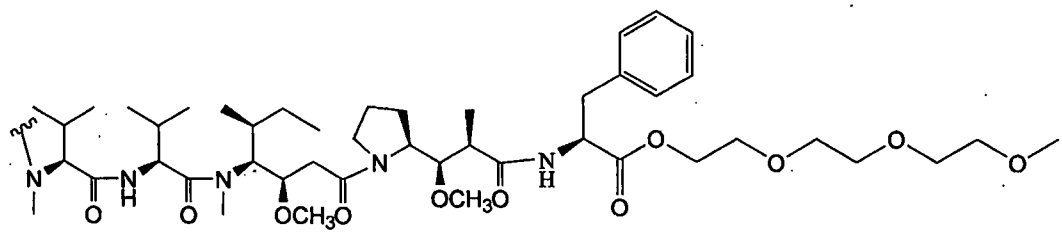
An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate (*see* US 2005/0238649 and Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124):

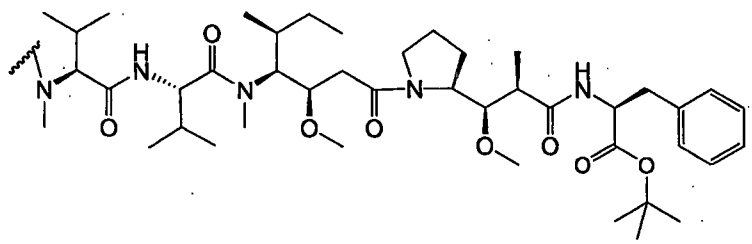
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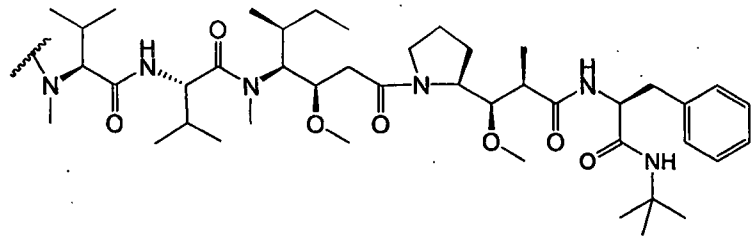


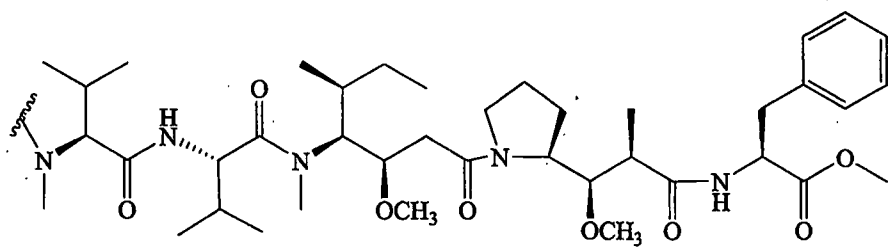
MMAF

Other drug moieties include the following MMAF derivatives, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

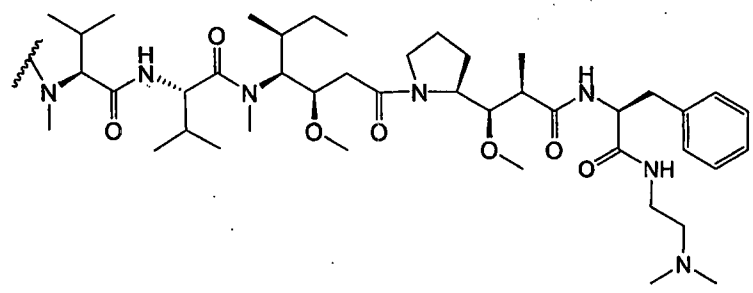




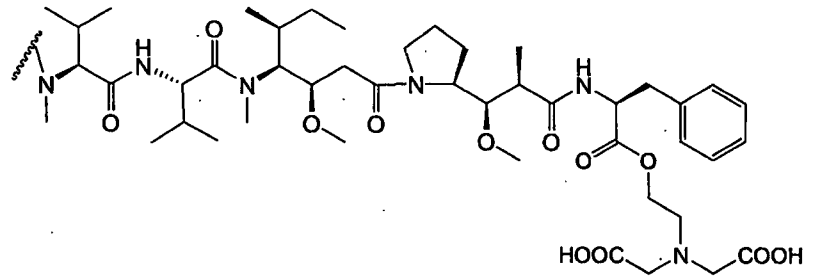




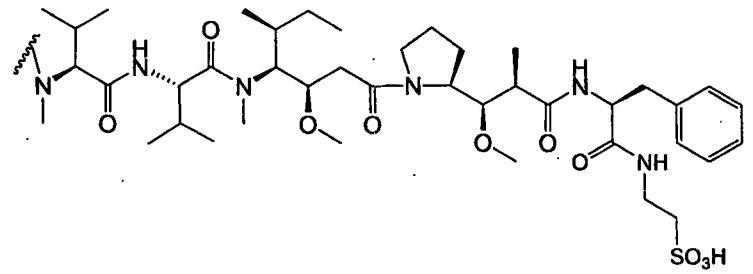
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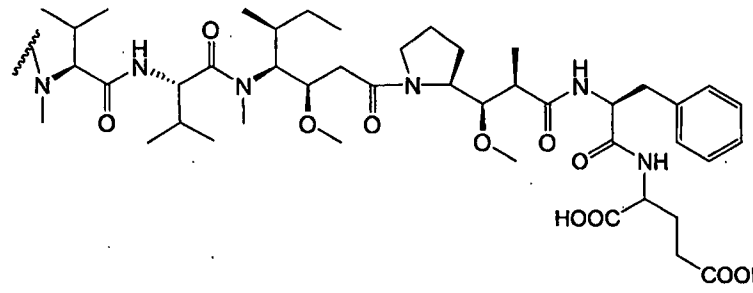
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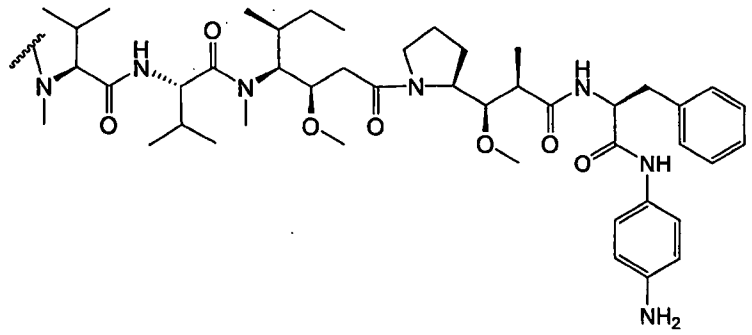


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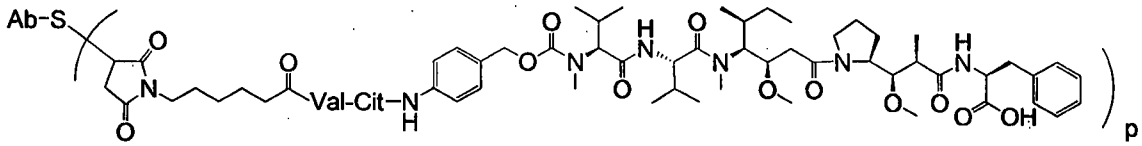
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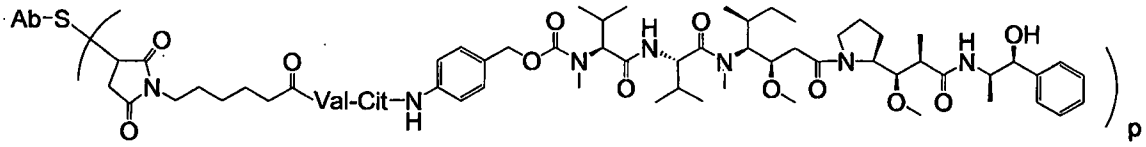


In one aspect, hydrophilic groups including but not limited to, triethylene glycol esters (TEG), as shown above, can be attached to the drug moiety at R^{11}. Without being bound by any particular theory, the hydrophilic groups assist in the internalization and non-agglomeration of the drug moiety.

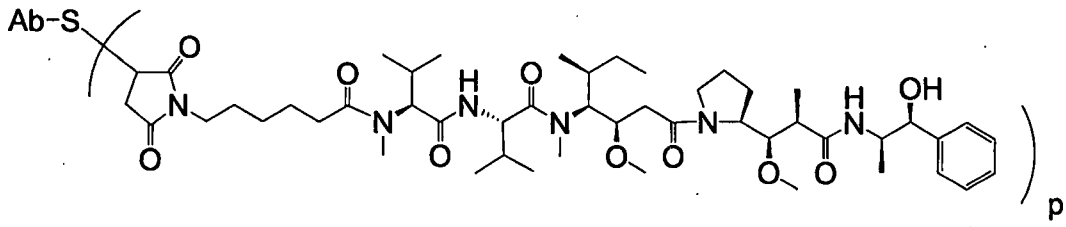
Exemplary embodiments of ADCs of Formula I comprising an auristatin/dolastatin or derivative thereof are described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124, which is expressly incorporated herein by reference. Exemplary embodiments of ADCs of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein "Ab" is an antibody; p is 1 to about 8, "Val-Cit" is a valine-citrulline dipeptide; and "S" is a sulfur atom:



Ab-MC-vc-PAB-MMAF

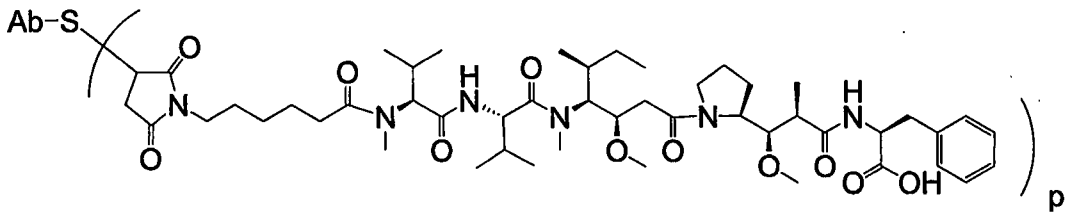


Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE

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Ab-MC-MMAF

Exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Interestingly, immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker. See, Doronina et al. (2006) Bioconjugate Chem. 17:114-124. In such instances, drug release is believed to be effected by antibody degradation in the cell. Id.

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. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. Auristatin/dolastatin drug moieties may be prepared according to the methods of: US 2005-0238649 A1; US Pat. No.5635483; US Pat. No.5780588; 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.

In particular, auristatin/dolastatin drug moieties of formula D_F, such as MMAF and derivatives thereof, may be prepared using methods described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124. Auristatin/dolastatin drug moieties of formula D_E, such as MMAE and derivatives thereof, may be prepared using methods described in Doronina et al. (2003) Nat. Biotech. 21:778-784. Drug-linker moieties MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE may be conveniently synthesized by routine methods, e.g., as described in Doronina et al. (2003) Nat. Biotech. 21:778-784, and Patent Application Publication No. US 2005/0238649 A1, and then conjugated to an antibody of interest.

Drug Loading

Drug loading is represented by p and is the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of

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Formula I 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, ELISA assay, and HPLC. 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 reverse phase HPLC or electrophoresis.

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).

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.

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

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linker-drug attachements (such as thioMab or thioFab prepared as disclosed herein and in WO2006/034488 (herein incorporated by reference in its entirety)).

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 (see, e.g., Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Certain Methods of Preparing Immunconjugates

An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in US 20050238649 A1, which is expressly incorporated herein by reference.

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine

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bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic subsituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

Nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A; see pages 467-498, 2003-2004 Applications Handbook and Catalog.

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Immunoconjugates comprising an antibody and a cytotoxic agent may also be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

Alternatively, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

Pharmaceutical Formulations

Administration of Antibody-Drug Conjugates

The antibody-drug conjugates (ADC) of the invention may be administered by any route appropriate to the condition to be treated. The ADC will typically be administered parenterally, i.e. infusion, subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural.

For treating cancers of, for example, prostate, lung and/or colon, in one embodiment, the antibody-drug conjugate is administered via intravenous infusion. The dosage administered via infusion is in the range of about 1 $\mu g/m^2$ to about 10,000 $\mu g/m^2$ per dose, generally one dose per week for a total of one, two, three or four doses. Alternatively, the dosage range is of about 1 $\mu g/m^2$ to about 1000 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 800 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 600 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 400 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 500 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 300 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 200 $\mu g/m^2$, and about 1 $\mu g/m^2$ to about 200 $\mu g/m^2$. The dose may be administered once per day, once per week, multiple

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times per week, but less than once per day, multiple times per month but less than once per day, multiple times per month but less than once per week, once per month or intermittently to relieve or alleviate symptoms of the disease. Administration may continue at any of the disclosed intervals until remission of the tumor or symptoms of the lymphoma, leukemia being treated. Administration may continue after remission or relief of symptoms is achieved where such remission or relief is prolonged by such continued administration.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding embodiments, which antibody is not conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding embodiments, which antibody is conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

In one aspect, the invention further provides pharmaceutical formulations comprising at least one anti-STEAP-1 antibody of the invention and/or at least one immunoconjugate thereof and/or at least one anti-STEAP-1 antibody-drug conjugate of the invention. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an anti-STEAP-1 antibody-drug conjugate and/or an immunoconjugate thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an immunoconjugate thereof, and optionally, 2) at least one additional therapeutic agent.

Pharmaceutical formulations comprising an antibody or immunoconjugate of the invention or the antibody-drug conjugate of the invention are prepared for storage by mixing the antibody or antibody-drug conjugate having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)) in the form of aqueous solutions or lyophilized or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, histidine 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

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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). Pharmaceutical formulations to be used for in vivo administration are generally sterile. This is readily accomplished by filtration through sterile filtration membranes.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate of the invention, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. 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. When encapsulated antibodies or immunoconjugates remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, 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.

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Antibody-Drug Conjugate Treatments

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders. Still others include prostate, lung and colon cancers.

The ADC compounds which are identified in the animal models and cell-based assays can be further tested in tumor-bearing higher primates and human clinical trials. Human clinical trials can be designed to test the efficacy of the anti-STEAP-1 monoclonal antibody or immunoconjugate of the invention in patients experiencing a prostate, lung or colon cell proliferative disorder including without limitation prostate, lung and colon cancers and metastases of such cancers. The clinical trial may be designed to evaluate the efficacy of an ADC in combinations with known therapeutic regimens, such as radiation and/or chemotherapy involving known chemotherapeutic and/or cytotoxic agents.

The cancer may comprise STEAP-1-expressing cells, such that an ADC of the present invention is able to bind to the cancer cells. To determine STEAP-1 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, STEAP-1 overexpression may be analyzed by IHC. Parrafin-embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a STEAP-1 protein staining intensity criteria with respect to the degree of staining and in what proportion of tumor cells examined.

For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight.

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For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of an anti-STEAP-1 antibody. Other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Combination Therapy

An antibody-drug conjugate (ADC) of the invention may be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with at least one additional compound having anti-cancer properties. The at lelast one additional compound of the pharmaceutical combination formulation or dosing regimen preferably has complementary activities to the ADC of the combination such that they do not adversely affect each other.

The at least one additional compound may be a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. A pharmaceutical composition containing an ADC of the invention may also have a therapeutically effective amount of a chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, or a DNA binder.

In one aspect, the first compound is an anti-STEAP-1 ADC of the invention and the at least one additional compound is a therapeutic antibody other than an anti-STEAP-1 (naked antibody or an ADC). In one embodiment, the at least one additional compound is an anti-PSCA antibody. In one embodiment the at least one additional compound is an anti-HER2 antibody, trastuzumab (e.g., Herceptin®, Genentech, Inc., South San Francisco, CA). In one embodiment the at least one additional compound is an anti-HER2 antibody, pertuzumab (Omnitarg™, Genentech, Inc., South San Francisco, CA, see US6949245). In one embodiment, the at least one additional compound is an anti-VEGF antibody (e.g, Avastin®, Genentech, Inc.). In each case, the at least one compound is either a naked antibody or an ADC). In an embodiment, the at least one additional compound is an antibody (either a naked antibody or an ADC), and the additional antibody is a second, third, fourth, fifth, sixth antibody or more, such that a combination of such second, third, fourth, fifth, sixth, or more antibodies (either naked or as an ADC) is efficacious in treating a cell proliferative disease in a tissue expressing STEAP-1.

Other therapeutic regimens may be combined with the administration of an anticancer agent identified in accordance with this invention, including without limitation radiation therapy and/or bone marrow and peripheral blood transplants, and/or a cytotoxic agent, a chemotherapeutic agent, or a growth

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inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA, anti-HER2 (e.g., Herceptin®, Omnitarg™) or anti-VEGF (e.g., Avastin®). The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one embodiment, treatment with an ADC involves the combined administration of an anticancer agent identified herein, and one or more chemotherapeutic agents or growth inhibitory agents, including coadministration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include taxanes (such as paclitaxel and docetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in "Chemotherapy Service", (1992) Ed., M.C. Perry, Williams & Wilkins, Baltimore, Md.

Suitable dosages for any of the above coadministered agents are those presently used and may be lowered due to the combined action (synergy) of the newly identified agent and other chemotherapeutic agents or treatments.

The combination therapy may provide "synergy" and prove "synergistic", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Metabolites of the Antibody-Drug Conjugates

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Also falling within the scope of this invention are the in vivo metabolic products of the ADC compounds described herein, to the extent such products are novel and unobvious over the prior art. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification, enzymatic cleavage, and the like, of the administered compound. Accordingly, the invention includes novel and unobvious compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof.

Metabolite products typically are identified by preparing a radiolabelled (e.g. 14C or 3H) ADC, administering it parenterally in a detectable dose (e.g. greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g. by MS, LC/MS or NMR analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found in vivo, are useful in diagnostic assays for therapeutic dosing of the ADC compounds of the invention.

Further Methods of Using Anti-STEAP-1 Antibodies and Immunoconjugates

Diagnostic methods and methods of detection

In one aspect, anti-STEAP-1 antibodies and immunoconjugates of the invention are useful for detecting the presence of STEAP-1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue. In certain embodiments, such tissues include normal and/or cancerous tissues that express STEAP-1 at higher levels relative to other tissues, for example, prostate, lung and colon.

In one aspect, the invention provides a method of detecting the presence of STEAP-1 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1.

In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of STEAP-1. In certain embodiments, the method comprises contacting a test cell with an anti-STEAP-1 antibody; determining the level of expression (either quantitatively or qualitatively) of STEAP-1

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by the test cell by detecting binding of the anti-STEAP-1 antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell (e.g., a normal cell of the same tissue origin as the test cell or a cell that expresses STEAP-1 at levels comparable to such a normal cell), wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of STEAP-1. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of STEAP-1. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor.

Exemplary cell proliferative disorders that may be diagnosed using an antibody of the invention include a prostate, lung and colon cancers or metastases of such cancers.

In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface. In certain embodiments, the method comprises contacting a cell with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1 on the cell surface. An exemplary assay for detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed STEAP-1 on the surface of a cell is a "FACS" assay.

Certain other methods can be used to detect binding of anti-STEAP-1 antibodies to STEAP-1. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

In certain embodiments, anti-STEAP-1 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-

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phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

In certain embodiments, anti-STEAP-1 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-STEAP-1 antibody from any STEAP-1 that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-STEAP-1 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al.., U.S. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-STEAP-1 antibody after formation of a complex between the anti-STEAP-1 antibody and STEAP-1, e.g., by immunoprecipitation.

Any of the above embodiments of diagnosis or detection may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-STEAP-1 antibody.

Therapeutic methods

An antibody or immunoconjugate of the invention may be used in, for example, in vitro, ex vivo, and in vivo therapeutic methods. In one aspect, the invention provides methods for inhibiting cell growth or proliferation, either in vivo or in vitro, the method comprising exposing a cell to an anti-STEAP-1 antibody or immunoconjugate thereof under conditions permissive for binding of the immunoconjugate to STEAP-1. "Inhibiting cell growth or proliferation" means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death. In certain embodiments, the cell is a tumor cell. In certain embodiments, the cell is a prostate, lung, colon, bladder, or ovary cell, or Ewing's sarcoma cell. In certain embodiments, the cell is a xenograft, e.g., as exemplified herein.

In one aspect, an antibody or immunoconjugate of the invention is used to treat or prevent a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder. In certain embodiments, the cell proliferative disorder is associated with increased expression and/or activity of STEAP-1. For example, in certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell. In certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is a tumor or a cancer or metastasis of such cancer.

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In one aspect, the invention provides methods for treating a prostate, lung or colon cell proliferative disorder comprising administering to an individual an effective amount of an anti-STEAP-1 antibody or immunoconjugate thereof. In certain embodiments, a method for treating a prostate, lung or colon cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising an anti-STEAP-1 antibody or anti-STEAP-1 immunoconjugate and, optionally, at least one additional therapeutic agent, such as those provided herein.

In one aspect, at least some of the antibodies or immunoconjugates of the invention can bind STEAP-1 from species other than human. Accordingly, antibodies or immunoconjugates of the invention can be used to bind STEAP-1, e.g., in a cell culture containing STEAP-1, in humans, or in other mammals having a STEAP-1 with which an antibody or immunoconjugate of the invention cross-reacts (e.g. chimpanzee, baboon, marmoset, cynomolgus and rhesus monkeys, dog, pig, rat, or mouse). In one embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used for targeting STEAP-1 on prostate, lung or colono cells by contacting the antibody or immunoconjugate with STEAP-1 to form an antibody or immunoconjugate-antigen complex such that a conjugated cytotoxin of the immunoconjugate accesses the interior of the cell. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is human STEAP-1. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is cynomolgus monkey STEAP-1. In one embodiment, the humanized anti-STEAP-1 antibody binds to human and/or cynomolgus monkey STEAP-1.

In one embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used in a method for binding STEAP-1 in an individual suffering from a disorder associated with increased STEAP-1 expression and/or activity, the method comprising administering to the individual the antibody or immunoconjugate such that STEAP-1 in the individual is bound. In one embodiment, the bound antibody or immunoconjugate is internalized into the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell expressing STEAP-1. In one embodiment, the STEAP-1 is human STEAP-1, and the individual is a human individual. Alternatively, the individual can be a mammal expressing STEAP-1 to which an anti-STEAP-1 antibody binds. Still further the individual can be a mammal into which STEAP-1 has been introduced (e.g., by administration of STEAP-1 or by expression of a transgene encoding STEAP-1).

An anti-STEAP-1 antibody or immunoconjugate can be administered to a human for therapeutic purposes. Moreover, an anti-STEAP-1 antibody or immunoconjugate can be administered to a non-human mammal expressing STEAP-1 with which the antibody cross-reacts (e.g., a primate, dog, pig, rat, or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal

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models may be useful for evaluating the therapeutic efficacy of antibodies or immunoconjugates of the invention (e.g., testing of dosages and time courses of administration).

Antibodies or immunoconjugates of the invention can be used either alone or in combination with other compositions in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent and/or adjuvant. In certain embodiments, an additional therapeutic agent is a cytotoxic agent, a chemotherapeutic agent, or a growth inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA (see, for example, US6824780), anti-VEGF (e.g., Avastin®, Genentech, Inc.), anti-HER2 (e.g., Herceptin®, Omnitarg™ Genentech, Inc.), or anti-HER2 in combination with Taxol® (see, for example, BioWorld Today, November 17, 1999, page 1), wherein the combination therapy is useful in the treatment of cell proliferative disorders, cancers, and/or metastases of cancers of prostate, lung and/or colon.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

An antibody or immunoconjugate of the invention (and any additional therapeutic agent or adjuvant) can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody or immunoconjugate is suitably administered by pulse infusion, particularly with declining doses of the antibody or immunoconjugate. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate need not be, but is optionally formulated with one or more agents currently used to

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prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or immunoconjugate present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents, such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of antibody or immunoconjugate, the severity and course of the disease, whether the antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the discretion of the attending physician. The antibody or immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg (e.g. 0.1mg/kg-20mg/kg) of antibody or immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) of antibody or immunoconjugate may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Assays

Anti-STEAP-1 antibodies and immunoconjugates of the invention may be characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Activity assays

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In one aspect, assays are provided for identifying anti-STEAP-1 antibodies or immunoconjugates thereof having biological activity. Biological activity may include, e.g., the ability to inhibit cell growth or proliferation (e.g., "cell killing" activity), or the ability to induce cell death, including programmed cell death (apoptosis). Antibodies or immunoconjugates having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vitro. Assays for inhibition of cell growth or proliferation are well known in the art. Certain assays for cell proliferation, exemplified by the "cell killing" assays described herein, measure cell viability. One such assay is the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. See Crouch et al (1993) J. Immunol. Meth. 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). See Cree et al (1995) AntiCancer Drugs 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

Another assay for cell proliferation is the "MTT" assay, a colorimetric assay that measures the oxidation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by mitochondrial reductase. Like the CellTiter-Glo™ assay, this assay indicates the number of metabolically active cells present in a cell culture. See, e.g., Mosmann (1983) J. Immunol. Meth. 65:55-63, and Zhang et al. (2005) Cancer Res. 65:3877-3882.

In one aspect, an anti-STEAP-1 antibody is tested for its ability to induce cell death in vitro. Assays for induction of cell death are well known in the art. In some embodiments, such assays measure, e.g., loss of membrane integrity as indicated by uptake of propidium iodide (PI), trypan blue (see Moore et al. (1995) Cytotechnology, 17:1-11), or 7AAD. In an exemplary PI uptake assay, cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. Cells are seeded at a density of 3×10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is removed and replaced with fresh medium alone or

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medium containing various concentrations of the antibody or immunoconjugate. The cells are incubated for a 3-day time period. Following treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Antibodies or immunoconjugates which induce statistically significant levels of cell death as determined by PI uptake are thus identified.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate is tested for its ability to induce apoptosis (programmed cell death) in vitro. An exemplary assay for antibodies or immunconjugates that induce apoptosis is an annexin binding assay. In an exemplary annexin binding assay, cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is removed and replaced with fresh medium alone or medium containing 0.001 to 10 μg/ml of the antibody or immunoconjugate. Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca^{2+} binding buffer, and aliquoted into tubes as discussed in the preceding paragraph. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 μg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (BD Biosciences). Antibodies or immunoconjugates that induce statistically significant levels of annexin binding relative to control are thus identified. Another exemplary assay for antibodies or immunconjugates that induce apoptosis is a histone DNA ELISA colorimetric assay for detecting internucleosomal degradation of genomic DNA. Such an assay can be performed using, e.g., the Cell Death Detection ELISA kit (Roche, Palo Alto, CA).

Cells for use in any of the above in vitro assays include cells or cell lines that naturally express STEAP-1 or that have been engineered to express STEAP-1. Such cells include tumor cells that overexpress STEAP-1 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express STEAP-1 and cell lines that do not normally express STEAP-1 but have been transfected with nucleic acid encoding STEAP-1.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit tumor growth in vivo. In vivo model systems, such as xenograft models, can be used for such testing. In an exemplary xenograft system, human tumor cells are introduced into a suitably immunocompromised non-human animal, e.g., a SCID mouse. An

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antibody or immunoconjugate of the invention is administered to the animal. The ability of the antibody or immunoconjugate to inhibit or decrease tumor growth is measured. In certain embodiments of the above xenograft system, the human tumor cells are tumor cells from a human patient. Such cells useful for preparing xenograft models include human prostate, lung, or colon tumor cell lines, which include without limitation PC3 cells expressing exogenous STEAP-1, and cells naturally expressing STEAP-1 which include, without limitation, LnCAP cells (Southern Research Institute, Birmingham, AL), LuCAP 77 cells, and LuCAP35V cells (University of Washington, Seattle, WA). In certain embodiments, the human tumor cells are introduced into a suitably immunocompromised non-human animal by subcutaneous injection or by transplantation into a suitable site, such as a mammary fat pad.

Binding assays and other assays

In one aspect, an anti-STEAP-1 antibody is tested for its antigen binding activity. For example, in certain embodiments, an anti-STEAP-1 antibody is tested for its ability to bind to STEAP-1 expressed on the surface of a cell. A FACS assay may be used for such testing.

In one aspect, competition assays may be used to identify a monoclonal antibody that competes with 120 graft or humanized variants thereof, including without limitation, 120v.24 antibody for binding to STEAP-1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by 120 graft antibody, or humanized 120 graft antibody, including variant 120v.24 humanized anti-STEAP-1 antibody. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). Two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more.

In an exemplary competition assay, immobilized STEAP-1 is incubated in a solution comprising a first labeled antibody that binds to STEAP-1 (e.g., murine 120.545 antibody, 120 graft antibody, or humanized 120v.24 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to STEAP-1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized STEAP-1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to STEAP-1, excess unbound antibody is removed, and the amount of label associated with immobilized STEAP-1 is measured. If the amount of label associated with immobilized STEAP-1 is substantially reduced in the test sample relative to the control sample, then that indicates that

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the second antibody is competing with the first antibody for binding to STEAP-1. In certain embodiments, immobilized STEAP-1 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface.

In one aspect, purified anti-STEAP-1 antibodies can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino acid analysis, non-denaturing size exclusion high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography and papain digestion.

In one embodiment, the invention contemplates an altered antibody that possesses some but not all effector functions, which make it a desirable candidate for many applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In certain embodiments, the Fc activities of the antibody are measured to ensure that only the desired properties are maintained. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991). An example of an in vitro assay to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art.

EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: Preparation of Humanized anti-STEAP-1 antibodies

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Nucleic acid molecules encoding amino acid sequence variants of the antibody, antibody fragment, VL domain or VH domain are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody, antibody fragment, VL domain or VH domain. For example, libraries can be created by targeting VL accessible amino acid positions in VH, and optionally in one or more CDRs, for amino acid substitution with variant amino acids using the Kunkel method. See, for e.g., Kunkel et al., Methods Enzymol. (1987), 154:367-382 and the examples herein. Generation of randomized sequences is also described below in the Examples.

The sequence of oligonucleotides includes one or more of the designed codon sets for a particular position in a CDR (HVR) or FR region of a polypeptide of the invention. A codon set is a set of different nucleotide triplet sequences used to encode desired variant amino acids. Codon sets can be represented using symbols to designate particular nucleotides or equimolar mixtures of nucleotides as shown in below according to the IUB code.

IUB CODES

G Guanine
A Adenine
T Thymine
C Cytosine
R (A or G)
Y (C or T)
M (A or C)
K (G or T)
S (C or G)
W (A or T)
H (A or C or T)
B (C or G or T)
V (A or C or G)
D (A or G or T)
N (A or C or G or T)

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For example, in the codon set DVK, D can be nucleotides A or G or T; V can be A or G or C; and K can be G or T. This codon set can present 18 different codons and can encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys.

Oligonucleotide or primer sets can be synthesized using standard methods. A set of oligonucleotides can be synthesized, for example, by solid phase synthesis, containing sequences that represent all possible combinations of nucleotide triplets provided by the codon set and that will encode the desired group of amino acids. Synthesis of oligonucleotides with selected nucleotide "degeneracy" at certain positions is well known in that art. Such sets of nucleotides having certain codon sets can be synthesized using commercial nucleic acid synthesizers (available from, for example, Applied Biosystems, Foster City, CA), or can be obtained commercially (for example, from Life Technologies, Rockville, MD). Therefore, a set of oligonucleotides synthesized having a particular codon set will typically include a plurality of oligonucleotides with different sequences, the differences established by the codon set within the overall sequence. Oligonucleotides, as used according to the invention, have sequences that allow for hybridization to a variable domain nucleic acid template and also can include restriction enzyme sites for cloning purposes.

In one method, nucleic acid sequences encoding variant amino acids can be created by oligonucleotide-mediated mutagenesis. This technique is well known in the art as described by Zoller et al, 1987, Nucleic Acids Res. 10:6487-6504. Briefly, nucleic acid sequences encoding variant amino acids are created by hybridizing an oligonucleotide set encoding the desired codon sets to a DNA template, where the template is the single-stranded form of the plasmid containing a variable region nucleic acid template sequence. After hybridization, DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will contain the codon sets as provided by the oligonucleotide set.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation(s). This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al., Proc. Nat'l. Acad. Sci. USA, 75:5765 (1978).

The DNA template is generated by those vectors that are either derived from bacteriophage M13 vectors (the commercially available M13mp18 and M13mp19 vectors are suitable), or those vectors that contain a single-stranded phage origin of replication as described by Viera et al., Meth. Enzymol., 153:3

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(1987). Thus, the DNA that is to be mutated can be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., above.

To alter the native DNA sequence, the oligonucleotide is hybridized to the single stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually T7 DNA polymerase or the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of gene 1, and the other strand (the original template) encodes the native, unaltered sequence of gene 1. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as E. coli JM101. After growing the cells, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabelled with a 32-Phosphate to identify the bacterial colonies that contain the mutated DNA.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTT), is combined with a modified thiodeoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell.

As indicated previously the sequence of the oligonucleotide set is of sufficient length to hybridize to the template nucleic acid and may also, but does not necessarily, contain restriction sites. The DNA template can be generated by those vectors that are either derived from bacteriophage M13 vectors or vectors that contain a single-stranded phage origin of replication as described by Viera et al. ((1987) Meth. Enzymol., 153:3). Thus, the DNA that is to be mutated must be inserted into one of these vectors in order

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to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., supra.

According to another method, a library can be generated by providing upstream and downstream oligonucleotide sets, each set having a plurality of oligonucleotides with different sequences, the different sequences established by the codon sets provided within the sequence of the oligonucleotides. The upstream and downstream oligonucleotide sets, along with a variable domain template nucleic acid sequence, can be used in a polymerase chain reaction to generate a "library" of PCR products. The PCR products can be referred to as "nucleic acid cassettes", as they can be fused with other related or unrelated nucleic acid sequences, for example, viral coat proteins and dimerization domains, using established molecular biology techniques.

Oligonucleotide sets can be used in a polymerase chain reaction using a variable domain nucleic acid template sequence as the template to create nucleic acid cassettes. The variable domain nucleic acid template sequence can be any portion of the heavy immunoglobulin chains containing the target nucleic acid sequences (ie., nucleic acid sequences encoding amino acids targeted for substitution). The variable region nucleic acid template sequence is a portion of a double stranded DNA molecule having a first nucleic acid strand and complementary second nucleic acid strand. The variable domain nucleic acid template sequence contains at least a portion of a variable domain and has at least one CDR. In some cases, the variable domain nucleic acid template sequence contains more than one CDR. An upstream portion and a downstream portion of the variable domain nucleic acid template sequence can be targeted for hybridization with members of an upstream oligonucleotide set and a downstream oligonucleotide set.

A first oligonucleotide of the upstream primer set can hybridize to the first nucleic acid strand and a second oligonucleotide of the downstream primer set can hybridize to the second nucleic acid strand. The oligonucleotide primers can include one or more codon sets and be designed to hybridize to a portion of the variable region nucleic acid template sequence. Use of these oligonucleotides can introduce two or more codon sets into the PCR product (i.e., the nucleic acid cassette) following PCR. The oligonucleotide primer that hybridizes to regions of the nucleic acid sequence encoding the antibody variable domain includes portions that encode CDR residues that are targeted for amino acid substitution.

The upstream and downstream oligonucleotide sets can also be synthesized to include restriction sites within the oligonucleotide sequence. These restriction sites can facilitate the insertion of the nucleic acid cassettes (i.e., PCR reaction products) into an expression vector having additional antibody sequence. In one embodiment, the restriction sites are designed to facilitate the cloning of the nucleic acid cassettes

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without introducing extraneous nucleic acid sequences or removing original CDR or framework nucleic acid sequences.

Nucleic acid cassettes can be cloned into any suitable vector for expression of a portion or the entire light or heavy chain sequence containing the targeted amino acid substitutions generated via the PCR reaction. According to methods detailed in the invention, the nucleic acid cassette is cloned into a vector allowing production of a portion or the entire light or heavy chain sequence fused to all or a portion of a viral coat protein (i.e., creating a fusion protein) and displayed on the surface of a particle or cell. While several types of vectors are available and may be used to practice this invention, phagemid vectors are the preferred vectors for use herein, as they may be constructed with relative ease, and can be readily amplified. Phagemid vectors generally contain a variety of components including promoters, signal sequences, phenotypic selection genes, origin of replication sites, and other necessary components as are known to those of ordinary skill in the art.

When a particular variant amino acid combination is to be expressed, the nucleic acid cassette contains a sequence that is able to encode all or a portion of the heavy or light chain variable domain, and is able to encode the variant amino acid combinations. For production of antibodies containing these variant amino acids or combinations of variant amino acids, as in a library, the nucleic acid cassettes can be inserted into an expression vector containing additional antibody sequence, for example all or portions of the variable or constant domains of the light and heavy chain variable regions. These additional antibody sequences can also be fused to other nucleic acids sequences, such as sequences that encode viral coat proteins and therefore allow production of a fusion protein.

Humanization of murine anti-human STEAP-1 antibody is described herein.

Materials and Methods

Residue numbers are according to Kabat (Kabat *et al., Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Single letter amino acid abbreviations are used. DNA degeneracies are represented using the IUB code (N = A/C/G/T, D = A/G/T, V = A/C/G, B= C/G/T, H= A/C/T, K = G/T, M = A/C, R = A/G, S = G/C, W= A/T, Y = C/T).

Cloning of murine 120 variable domains and generation of a chimeric 120 antibody – Total RNA was extracted from hybridoma cells producing M2-120.545 (designated as "murine 120" or "mu120" herein) using standard methods. The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers to the heavy and light chains. The forward primers were specific for the N-terminal amino acid sequence of the VL and VH regions. Respectively, the LC and HC reverse

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primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which are highly conserved across species. Amplified VL and VH were cloned into mammalian expression vectors. The polynucleotide sequence of the inserts was determined using routine sequencing methods. The M2-120.545 ("mu 120") VL and VH amino acid sequences are shown in Figures 2A and 2B, respectively.

Generation of murine 120 chimera – A chimeric anti-STEAP-1 antibody was prepared by fusing the murine 120 variable heavy (VH) and variable light (VL) regions to the constant domains of a human IgG. The resultant antibody is designated "120 chimera," "chimera 120," "chimeric 120 IgG," or "Fc chimera" herein.

Direct hypervariable region grafts onto the acceptor human consensus framework – Variants constructed during the humanization of murine 120 were assessed both as protein in the form of an IgG or as a Fab displayed on phage.

The phagemid used for this work is a monovalent Fab-g3 display vector and consists of two open reading frames under control of the phoA promoter. The first open reading frame consists of the stII signal sequence fused to the VL and CH1 domains of the acceptor light chain and the second consists of the stII signal sequence fused to the VH and CH1 domains of the acceptor heavy chain followed by the minor phage coat protein P3.

The VL and VH domains from murine 120 were aligned with the human VL kappa I (huKI) and human VH subgroup III (huIII) consensus sequences. To make the CDR grafts, hypervariable regions from the murine 120 antibody were grafted into the huKI and huIII acceptor frameworks.

Hypervariable regions from murine 120 antibody (mu120) were engineered into the acceptor human consensus framework to generate the direct CDR-graft (designated as "120 graft," or "graft 120" herein). In the VL domain the following regions were grafted to the human consensus acceptor: positions 24-34 (L1), 50-56 (L2) and 89-97 (L3). In the VH domain, positions 26-35a (H1), 49-65 (H2) and 95-102 (H3) were grafted. The sequences of the light and heavy chain variable regions of the 120 graft are shown in Figures 2A-2B. The CDRs (also designated here as HVRs) are shown in boxes (Figures 2A-2B). These CDR definitions include positions defined by their sequence hypervariability (Kabat ref), their structural location (Chothia ref) and their involvement in antigen-antibody contacts (MacCallum *et al. J. Mol. Biol.* 262: 732-745 (1996)).

The direct-graft variants expressed as a Fab displayed on phage or as an IgG were generated by Kunkel mutagenesis using a separate oligonucleotide for each hypervariable region. Correct clones were assessed by DNA sequencing.

Generation of humanized 120 phage variants – Humanized 120 variants were generated as Fab displayed on phage by Kunkle mutagenesis. A phosphorylated oligonucleotide was added to 300 ng

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Kunkel template in 50 mM Tris pH 7.5, 10 mM $MgCl_2$ in a final volume of 10 µl. The mixture was annealed at 90 °C for 2 min, 50 °C for 5 min and then cooled on ice. The annealed template was then filled in by adding 0.5 µl 10mM ATP, 0.5 µl 10mM dNTPs (10mM each of dATP, dCTP, dGTP and dTTP), 1 µl 100mM DTT, 1 µl 10X TM buffer (0.5 M Tris pH 7.5, 0.1 M $MgCl_2$), 80 U T4 ligase, and 4 U T7 polymerase in a total volume of 20 µl for 2 h at room temperature. The filled in and ligated product was then transformed into XL1-blue cells (Stratagene). Correct clones were identified by DNA sequencing.

Correct phage clones were grown in 25 ml of 2YT containing 50 µg/ml carbenacillin and M13/KO7 helper phage (MOI 10) overnight at 37 °C.

Assessment of humanized 120 variants – Humanized variants expressed as IgG were assessed by FACS analysis using Steap1 positive (293 Steap1 NT LB50) and negative (293 vector S408) cell lines. Humanized variants expressed as a Fab displayed on phage were also assessed by FACS analysis. Phage expressing Fab variants were first assessed for their Fab display level using an phage ELISA used to detect a flag-tag fused to the light chain of the Fab. MaxiSorp microtiter plates were coated with anti-gD 1766 at 10 µg/ml in PBS over night and then blocked with Casein Blocker. Phage from culture supernatants were serially diluted in PBST containing 0.5 % BSA in a tissue culture microtiter plate and transferred to the coated wells for 1 h to capture the Fab displaying phage. The plate was washed with PBST and HRP conjugated anti-M13 (Amersham Pharmacia Biotech) was added (1:5000 in PBST containing 0.5 % BSA) for 40 min. The plate was washed with PBST and developed by adding Tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The absorbance at 405 nm was used as an estimate of the Fab display level on the surface of the phage. Phage preparations were normalized for display by dilution. Low displaying phage (e.g. the chimera) were used neat for FACS analysis.

For FACS analysis of phage binding, cells were removed from the plate using 2 mM EDTA, collected in a 15 mL conical bottom tube and pelleted by centrifugation. Cells (5 X 10^5 cells per sample) were re-suspended in 100 µL of phage (normalized by display level) in FACS buffer (1% FBS, PBS with 2 mM EDTA) and incubated for 1-2 hours on ice. Samples were washed twice with FACS buffer by centrifugation. Anti-M13 5G7 control antibody (Genentech, Inc. South San Francisco, CA) was added at 2 µg/mL and incubated on ice for at least 45 minutes. Samples were washed twice with FACS buffer by centrifugation. A 1:200 dilution of anti-mouse PE (R-phycoerythrin goat anti-mouse IgG Fcy Fragment, Jackson Immunoresearch) was added and incubated on ice for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and analyzed by FACS.

For analysis of IgG by FACS, cells were prepared as in the phage FACS. Each IgG was added at 5 µg/mL on ice for 1 hour. Samples were washed twice with FACS buffer by centrifugation and a 1:200 dilution of anti-human PE conjugate (R-phycoerythrin goat anti-human IgG Fcy Fragment, Jackson

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Immunoresearch) was added for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and samples were analyzed by FACS.

IgG Production and Affinity Determination -IgG was purified with Protein G affinity chromatography. Affinity determinations were performed by Scatchard analysis on 293 STEAP-1 NT LB50 cells.

Results and Discussion

Murine 120 variable domain sequences and CDR assignment graft design – The human acceptor framework used for humanization of M2-120.545 is based on the consensus human kappa I VL domain and the human subgroup III consensus VH domain. The VL and VH domains of murine M2-120.545 were each aligned with the human kappa I and subgroup III domains; each complimentarity region (CDR) was identified and grafted into the human acceptor framework to generate a CDR graft that could be displayed as an Fab on phage and expressed as an IgG. The sequences of humanized anti-STEAP-1 antibody version 24 variable regions are shown in Figures 2A and 2B. The 120-graft Fab displayed on phage and 120-graft IgG were tested for binding to exogenous STEAP-1 expressing cells (293 STEAP-1 NT LB50) by FACS analysis. Although the 120-graft IgG bound specifically to the STEAP-1 expressing cells, the FACS signal observed for the 120-graft IgG was smaller than that observed for the chimeric 120 IgG indicating a loss in binding affinity. Phage displaying the 120-graft Fab also generated a FACS signal that was only observed on STEAP-1 expressing cells. This shift was less than that observed for the chimeric 120 IgG. Scatchard analysis of the 120-graft IgG also indicated a significant (approximately 50-fold) loss in binding affinity (KD = 36 nM for the 120v.78; KD = 260 nM for 120 graft).

Humanization of M2-120.545 –Approximately 30 vernier positions that influence CDR conformation and VL:VH domain packing have been identified and changes at these positions between the donor and human frameworks should be considered when humanizing antibodies (Foote, J. and Winter, G., J. Mol. Biol.224(2):487-499 (1992)). An assessment of the alignment of murine M2-120.545 with the consensus human kappa I VL domain and the human subgroup III consensus VH domain revealed sequence differences at 6 key vernier positions in the VH domain: 24, 37, 48, 67, 73, 78 (see Figure 2B). To assess the influence of these positions, murine residues were individually introduced into the human consensus subgroup III VH domain of the Fab on phage. This involved making the following mutations to the 120-graft Fab displayed on phage individually: A24V (120.v24), V37I (120.v37), V48M (120.v48), F67I (120.v67), and L78F (120.v78). N73T was not tested. Each phage variant was normalized by dilution to an equivalent Fab display level determined by titration of an epitope tag fused to the light chain displayed on the phage and then assessed for binding to STEAP-1 by FACS analysis on STEAP-1-expressing cells (293

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STEAP-1 NT LB50) and non-expressing (293 vector S408) cells. The term "2°" refers to the secondary antibody in the FACS analysis. The term "α-120" refers to the murine 120 anti-STEAP-1 antibody. The term "α-10H1" refers to a control antibody. The terms "24 Phage," "37 Phage," and the like refer to humanized anti-STEAP-1 variants as disclosed herein displayed on phage. "Ch 120 Phage" refers to the 120 Chimera displayed on phage, and "120.graft Phage" refers to the 120 graft displayed on Phage. (Figure 6). The importance of normalizing phage clones by their Fab display level is illustrated by a FACS analysis of the 120-graft at different phage titers: $7x10^{12}$ phage/ml in Figure 6 and 2×10^{11} phage/ml in Figure 6. Once diluted to the lower phage concentration, the 120-graft phage no longer produced an observable FACS shift. Thus normalization of the different phage clones for their display level was important step for assessing their affinity differences for Steap1.

Following normalization for Fab display levels, the variant of the 120-graft containing the additional mutation A24V (120.v24) produced a FACS shift superior to other variants (Figure 6). When expressed as an IgG, 120.v24 produced a similar FACS shift to the chimeric 120 antibody at all concentrations tested. Subsequent Scatchard analysis of 120.v24 indicated a Kd of 2.2 nM for binding to 293 STEAP-1 NT LB50 cells, a two-fold improvement over the 120 chimera and the original murine M2-120.545 (Table 2).

Table 2: Anti-STEAP-1 antibody binding affinity for cell surface STEAP-1 (Kd (nM))

Cell line	Murine anti-STEAP-1 MAb 120.545 nM	120 Chimera	Humanized anti-STEAP-1 120v.24
PC3-PS5.4 (exogenous STEAP-1)	17.5 nM 187,256 sites per cell	9.9 nM 103,204 sites per cell	---
293.LB50 (exogenous STEAP-1)	4.7 nM 301,100 sites per cell	4.9 nM 252,892 sites per cell	2.2 nM 264,172 sites per cell
LNCaP-BR (endogenous STEAP-1)	1.5 nM 37,207 sites per cell	0.9 nM 22,021 sites per cell	---

Binding activity of anti-STEAP-1 naked antibodies, murine 120 and chimera 120 was also tested using FACS analysis. Binding was compared for exogenous STEAP-1 in 293 stable STEAP-1 NT LB50, PC3 stable STEAP-1 PS5.4, and endogenous STEAP-1 in LNCaP cells. The results are also shown in Figs. 7D-7F. NT LB50 cells expressing exogenous human STEAP-1 on the cell surface were prepared by stably transforming 293 cells (ATCC CRL-1573) with human STEAP-1 DNA. PS5.4 cells expressing

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exogenous human STEAP-1 on the cell surface were prepared by stably transforming PC3 (ATCC CLL-1435) with human STEAP-1 DNA. LNCaP cells (ATCC CRL-1740) express STEAP-1 endogenously.

Example 2: Characterization of anti-STEAP-1 antibodies

Anti-STEAP-1 antibodies (naked antibodies and antibody drug conjugates disclosed herein) were characterized or may be characterized according to standard methods.

ELISA-based assays: Anti-STEAP-1 antibody screening by ELISA is performed as follows, with all incubations done at room temperature. Test plates (Nunc Immunoplate) were coated for 2 hours with purified STEAP-1 in 50 mM sodium carbonate buffer, pH 9.6, then blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS) for 30 minutes, then washed four times with PBS containing 0.05% Tween 20 (PBST). Test antibody supernatants are added and incubated two hours with shaking, then washed four times with PBST. The plates are developed by adding 100 µl/well of a solution containing 10 mg of o-phenylenediamine dihydrochloride (Sigma, #P8287) and 10 µl of a 30% hydrogen peroxide solution in 25 ml phosphate citrate buffer, pH 5.0, and incubating for 15 minutes. The reaction is stopped by adding 100 µl/well of 2.5 M sulfuric acid. Data is obtained by reading the plates in an automated ELISA plate reader at an absorbance of 490 nm.

Characterization of anti-STEAP-1 binding by Scatchard analysis:

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980) using standard techniques well know in the relevant art. See also Scathcard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

Example 3: Production of Anti-STEAP-1 Antibody Drug Conjugates

Production of anti-STEAP-1 auristatin ADCs - Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies murine 120.545, 120 chimera, 120 graft, and humanized 120 framework variants to the following drug-linker moieties: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE, MC-MMAF, vc-MMAE, and vc-MMAF, which drug and linker moieties are disclosed herein as well as in WO 2004/010957, published February 5, 2004, and WO2006/034488, published September 9, 2005 (each of which patent applications is herein incorporated by reference in its entirety). Prior to conjugation, the antibodies were partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957. The partially reduced antibodies were conjugated to the above drug-linker moieties using standard methods in

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accordance with the methodology described in Doronina et al. (2003) Nat. Biotechnol. 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibodies were combined with the drug linker moieties to allow conjugation of the moieties to cysteine residues. The conjugation reactions were quenched, and the ADCs were purified. The drug load (average number of drug moieties per antibody) for each ADC was determined by HPLC. As used herein, the linker-drug component of an ADC, " –MC-vc-PAB-MMAE" or "-MC-vc-PAB-MMAF" is sometimes abbreviated as " –vcMMAE" or "-vcMMAF," and the component "-MC-MMAF" is sometimes abbreviated as "MCMMAF" or "mcMMAF."

Production of anti-STEAP-1 maytansinoid ADCs – Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies, murine 120, 120 chimera, 120 graft, and humanized 120 framework variants to the linker drug moiety –smcc-DM1. Such conjugation may be performed according to the method disclosed in WO 2005/037992 for conjugation of Herceptin® anti-HER2 antibody.

Example 4: In Vivo Tumor Volume Reduction Assay

To test the efficacy of toxin-conjugated or unconjugated anti-STEAP-1 monoclonal antibodies for the ability to reduce tumor volume in vivo, the following protocol was employed.

In vivo prostate cancer xenograft models were used to test the efficacy of anti-STEAP-1 ADCs. These models included human cell line LNCaP (ATCC CRL-1740 or Southern Research Institute, Birmingham, AL). Prostate explant models included LuCaP 77 and LuCaP35V (University of Washington, Seattle, WA). Each prostate explant model was maintained by serial transplanting in castrated (androgen independent model) or uncastrated (androgen dependent model), male SCID-beige mice from Charles River Lab. The uncastrated mice received a testosterone pellet prior to implantation, while castration was done at least 2 weeks prior to tumor implantation to allow testosterone levels to nadir. When donor mice have tumors of between 800-1000 mm^3, tumor tissue were aseptically removed and dissected into small implantable sized pieces (approximately 20 mm^3). The tumor is placed into a pocket at the implantation site and the skin is closed using wound clips. For the LNCaP cell line model, in vitro grown LNCaP cells were injected subcutaneously at 8-10 million cells per mouse in 50% matrigel into male SCID-beige mice which had received a testosterone pellet. When mean tumor size reached 100-200mm^3, animals were randomly grouped in 10 groups of 10 mice each and given a single IV administration of test antibody ADC or control antibody (naked or control). In some experiments, multiple doses of test or control antibody were administered (see Figures 8A, 9, and 10). In some experiments, a single dose of test and control antibody were administered as seen in Figures 8B and 11. Where the prostate explant model was LuCap 77, a testosterone pellet was implanted in the mice approximately 3-7 days before transplantation of exogenous tumor. Tumors were measured twice per week for 4 weeks, then

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once or twice per week for the remainder of the study or once per week throughout the study. A significantly lower tumor volume in test animals over time was considered to an indication of efficacy. In some cases, tumor volume decreased significantly from the initial volume and remained low throughout the study. Results are plotted in Figures 8-11.

Anti-STEAP-1 auristatin drug conjugates reduce prostate tumor volume in vivo

Administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. PBS and anti-gp120-MC-vc-PAB-MMAE (3 mg/kg) were used as controls. Doses were administered on days 0, 7, and 14. See Figure 8A.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg), and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) to SCID beige mice transplanted with LNCap-Ner tumor (treated with a testosterone pellet as described herein) was shown to be efficacious. Vehicle, anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (12 mg/kg) were used as controls. Doses were administered on the days indicated in Figure 8. The results are plotted in Figure 8B.

Administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) and anti-STEAP-1 120 chimera-MC-MMAF (6 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID-beige mice transplanted with LNCaP cells. Three doses at approximately days 15, 25, and 30 at 3 mg/kg (anti-STEAP-vcMMAE) or 6 mg/kg (anti-STEAP-mcMMAF) were administered to the mice. Control anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (6 mg/kg) were used. See Figure 9.

Administration of humanized anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. Controls were vehicle and anti-ragweed-MC-vc-PAB-MMAE. Three doses at 3 mg/kg of test and control antibodies were administered. See Figure 10.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. Drug load was 3.1 per antibody. Control antibodies were anti-ragweed-MC-MMAF administered at 12 mg/kg, and anti-gp120-MC-vc-PAB-MMAE administered at 6 mg/kg. See Figure 11.

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

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WHAT IS CLAIMED IS:

1. An antibody that binds to STEAP-1, wherein the antibody comprises at least one, two, or three heavy chain (HC) hypervariable regions (HVRs) selected from:
   (1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
   (2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and
   (3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

2. The antibody of claim 1, further comprising comprising at least one, two, or three HC framework regions (FRs) selected from:
   (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
   (2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
   (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
   (4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
   (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
   (6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
   (7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
   (8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

3. The antibody of claim 1, further comprising a light chain (LC) of SEQ ID NO:6.

4. The antibody of claim 2, further comprising a light chain (LC) of SEQ ID NO:6.

5. The antibody of claim 1, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

6. The antibody of claim 2, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

7. The antibody of claim 1, wherein the antibody is humanized.

8. The antibody of claim 2, wherein the antibody is humanized.

9. The antibody of claim 1, wherein the STEAP-1 is mammalian STEAP-1.

10. The antibody of claim 9, wherein the STEAP-1 is selected from rodent STEAP-1 and primate STEAP-1.

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11. The antibody of claim 31, wherein the STEAP-1 is human STEAP-1.

12. A polynucleotide encoding a HC comprising at least one, two, or three HC-HVRs selected from:
(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

13. A polynucleotide of claim 12 comprising at least one, two, three, or four HC-FRs selected from:
(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

14. A vector comprising the polynucleotide of claim 12.

15. A host cell comprising the vector of claim 12.

16. The host cell of claim 15, wherein the host cell is a bacteria, yeast, insect, or eukaryotic cell.

17. The host cell of claim 42, wherein the host cell is a CHO cell.

18. A method of making an anti-STEAP-1 antibody, wherein the method comprises a) culturing the host cell of claim 15 under conditions suitable for expression of the polynucleotide encoding the antibody, and b) isolating the antibody.

19. The antibody of claim 1, wherein the STEAP-1 is expressed on the surface of a cell.

20. The antibody of claim 19, wherein the cell is a prostate, lung, colon, bladder, or ovary cell or a Ewing's sarcoma cell.

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21. The antibody of claim 20, wherein the cell is associated with a proliferative disorder.

22. The antibody of claim 21, wherein the disorder is cancer.

23. The antibody of claim 22, wherein the cancer is metastasis.

24. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

25. The antibody of claim 24, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

26. The antibody of claim 60, wherein the antibody is humanized.

27. The antibody of claim 60, wherein the antibody is human.

28. A method of detecting the presence of STEAP-1 in a biological sample, the method comprising contacting the biological sample with an antibody of claim 1 under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1.

29. The method of claim 28, wherein the biological sample is from a patient suspected of having a prostate, lung, colon, bladder, ovarian cell or Ewing's sarcoma cell proliferative disorder.

30. An immunoconjugate comprising an antibody of claim 1 covalently attached to a cytotoxic agent.

31. The immunoconjugate of claim 30, wherein the cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

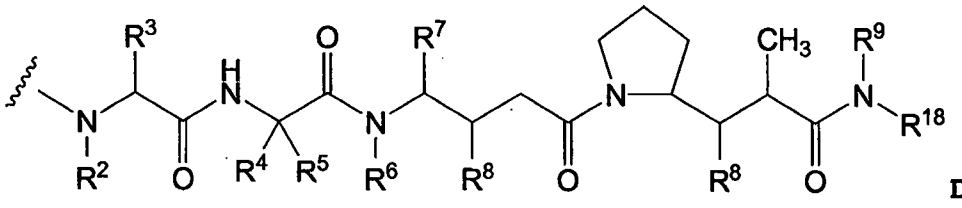
32. The immunoconjugate of claim 31, the immunoconjugate having the formula Ab-(L-D)p, wherein:
    (a) Ab is the antibody of claim 1;
    (b) L is a linker;
    (c) D is a drug moiety.

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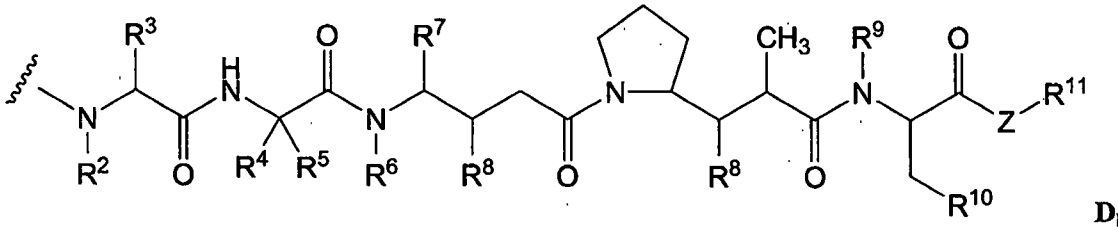
33. The immunoconjugate of claim 32, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenyloxycarbonyl (MC-vc-PAB).

34. The immunoconjugate of claim 33, wherein D is selected from an auristatin and dolostantin.

35. The immunoconjugate of claim 34, wherein D is a drug moiety of formula D_E or D_F:



D_E



D_F

and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $–(CH_2)_2–O–(CH_2)_2–O–(CH_2)_2–O–CH_3$; and R^{18} is $–C(R^8)_2–C(R^8)_2–$aryl; and

(d) p ranges from about 1 to 8.

36. The immunoconjugate of claim 32, having in vitro or in vivo cell killing activity.

37. The immunoconjugate of claim 32, wherein the linker is attached to the antibody through a thiol group on the antibody.

38. The immunoconjugate of claim 32, wherein the linker is cleavable by a protease.

39. The immunoconjugate of claim 32, wherein the linker comprises a val-cit dipeptide.

40. The immunoconjugate of claim 32, wherein the linker comprises a p-aminobenzyl unit.

41. The immunoconjugate of claim 32, wherein the linker comprises 6-maleimidocaproyl.

42. The immunoconjugate of claim 32, wherein the drug is selected from MMAE and MMAF.

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43. The immunoconjugate of claim 42, wherein the drug is MMAE.

44. The immunoconjugate of claim 42, wherein the drug is MMAF.

45. The immunoconjugate of claim 32, wherein the immunoconjugate has the formula

Ab-(L-MMAE)p,

wherein L is a linker and p ranges from 2 to 5.

46. The immunoconjugate of claim 45, wherein L comprises val-cit.

47. The immunoconjugate of claim 45, wherein L comprises MC.

48. The immunoconjugate of claim 45, wherein L comprises PAB.

49. The immunoconjugate of claim 45, wherein L comprises MC-vc-PAB.

50. The immunoconjugate of claim 45, wherein the immunoconjugate has the formula

Ab-(L-MMAF)p,

wherein L is a linker and p ranges from 2 to 5.

51. The immunoconjugate of claim 50, wherein L comprises val-cit.

52. The immunoconjugate of claim 50, wherein L comprises MC.

53. The immunoconjugate of claim 50, wherein L comprises PAB.

54. The immunoconjugate of claim 50, wherein L comprises MC-vc-PAB.

55. The immunoconjugate of claim 50, wherein the immunoconjugate has the formula

Ab-(L-MMAF)p,

wherein L is a linker and p ranges from 2 to 5.

56. The immunoconjugate of claim 32, wherein D is a maytansinoid.

57. The immunoconjugate of claim 56, wherein D is selected from DM1, DM3, and DM4.

58. The immunoconjugate of claim 56, having in vitro or in vivo cell killing activity.

59. The immunoconjugate of claim 56, wherein the linker is attached to the antibody through a thiol group on the antibody.

60. The immunoconjugate of claim 56, wherein the linker, L, is selected from N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

61. The immunoconjugate of claim 60, wherein the drug is DM1.

62. The immunoconjugate of claim 62, wherein L comprises a SPP.

63. The immunoconjugate of claim 61, wherein L comprises SMCC.

64. The immunoconjugate of claim 61, wherein p is from 2-6.

65. The immunoconjugate of claim 64, wherein p is from 3-5.

66. A pharmaceutical composition comprising the immunoconjugate of claim 32 and a pharmaceutically acceptable carrier.

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67. A method of treating a cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 66.

68. The method of claim 67, wherein the cell proliferative disorder is selected from prostate, lung, colon, bladder, ovary, and Ewing's sarcoma cell proliferative disorder.

69. A method of inhibiting prostate, lung, colon, bladder, ovary, Ewing's sarcoma cell proliferation comprising exposing a cell to an immunoconjugate of claim 32 under conditions permissive for binding of the immunoconjugate to STEAP-1.

70. The method of claim 69, wherein the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell is a xenograft.

71. The method of claim 69, wherein the exposing takes place in vitro.

72. The method of claim 69, wherein the exposing takes place in vivo.

73. The antibody of claim 1, wherein the antibody is a cysteine engineered antibody comprising one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0, wherein the cysteine engineered antibody is prepared by a process comprising replacing one or more amino acid residues of a parent antibody by cysteine.

74. The cysteine engineered antibody of claim 73, wherein the cysteine engineered antibody is more reactive than the parent antibody with a thio-reactive reagent.

75. The cysteine engineered antibody of claim 73 wherein the process further comprises determining the thiol reactivity of the cysteine engineered antibody by reacting the cysteine engineered antibody with a thiol-reactive reagent;

wherein the cysteine engineered antibody is more reactive than the parent antibody with the thiol-reactive reagent.

76. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a light chain.

77. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a heavy chain.

78. The cysteine engineered antibody of claim 73, wherein the antibody is an immunoconjugate comprising the cysteine engineered antibody covalently attached to a cytotoxic agent.

79. The cysteine engineered antibody of claim 78, wherein the cytotoxic agent is selected fom a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

80. The cysteine engineered antibody of claim 73 wherein the antibody is covalently attached to a capture label, a detection label, or a solid support.

81. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a biotin capture label.

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82. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a fluorescent dye detection label.

83. The cysteine engineered antibody of claim 82 wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

84. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a radionuclide detection label selected from ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi.

85. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a chelating ligand that chelates a detectable label.

86. The cysteine engineered antibody of claim 80 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

87. The immunoconjugate of claim 32, wherein the linker is attached to the antbody through a thiol group on the antibody.

88. The antibody of claim 1 comprising an albumin binding peptide.

89. The antibody of claim 88, wherein the albumin binding peptide is selected from SEQ ID NOs:80-84.

90. The method of claim 67, further comprising administering to the individual an effective amount of at least one additional therapeutic compound selected from a chemotherapeutic acid, a radionuclide, CHOP, a naked antibody, antibody drug conjugate, and an immunoconjugate.

91. The method of claim 90, wherein the antibody, ADC, and immunoconjugate bind to an antigen expressed in proliferating cells.

92. The method of claim 91, wherein the proliferating cells are from prostate, lung, colon, bladder, ovary, or Ewing's sarcoma.

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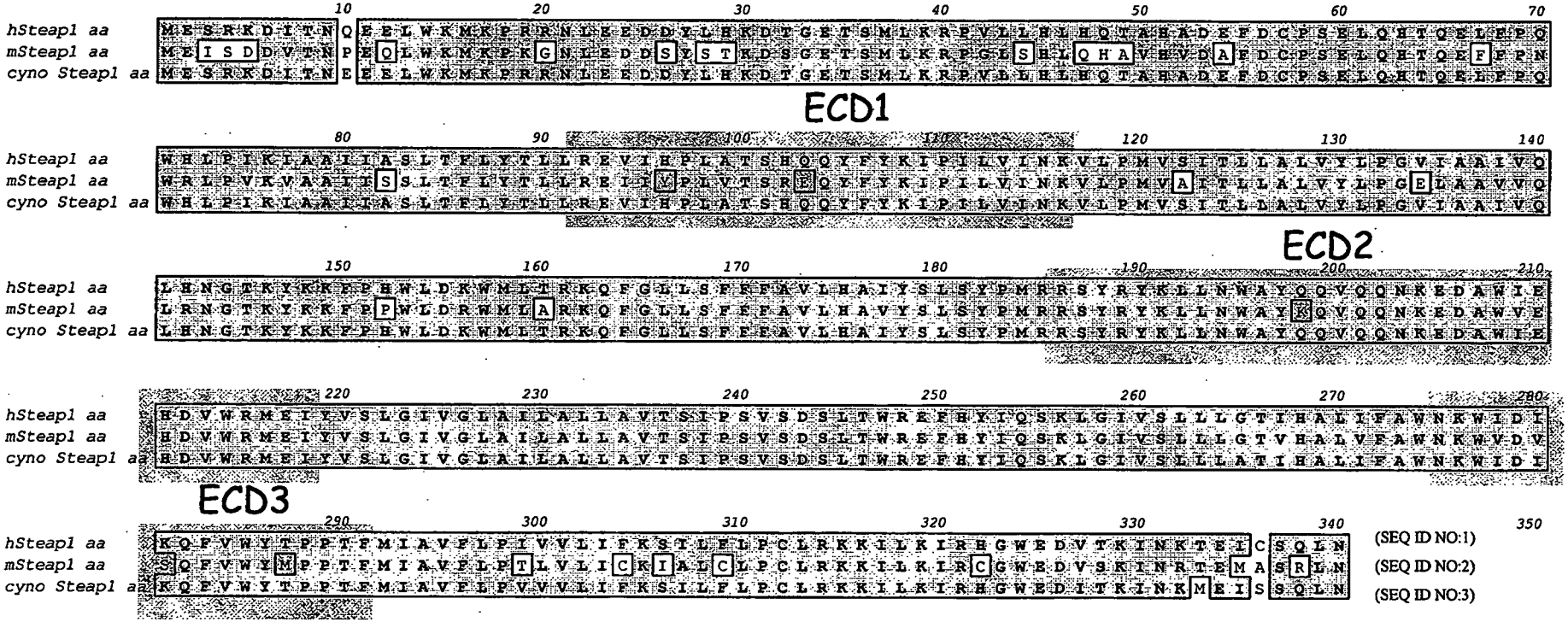
ABSTRACT OF THE DISCLOSURE

Anti-STEAP-1 antibodies and immunoconjugates thereof are provided. Methods of using anti-STEAP-1 antibodies and immunoconjugates thereof are provided.

5

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Figure 1



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Alignment of Light Chain Sequences

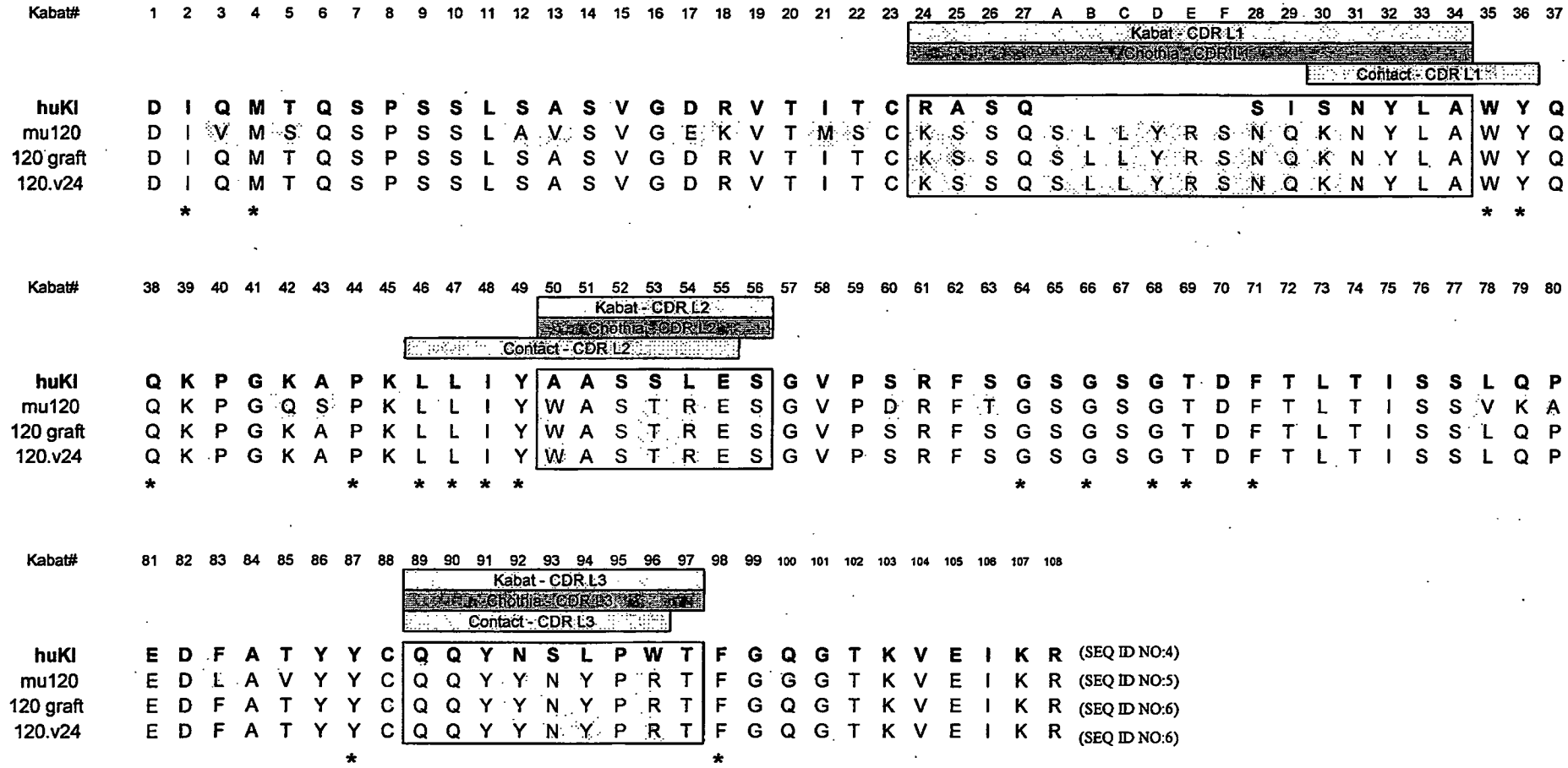


Figure 2A

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Alignment of Heavy Chain Sequences

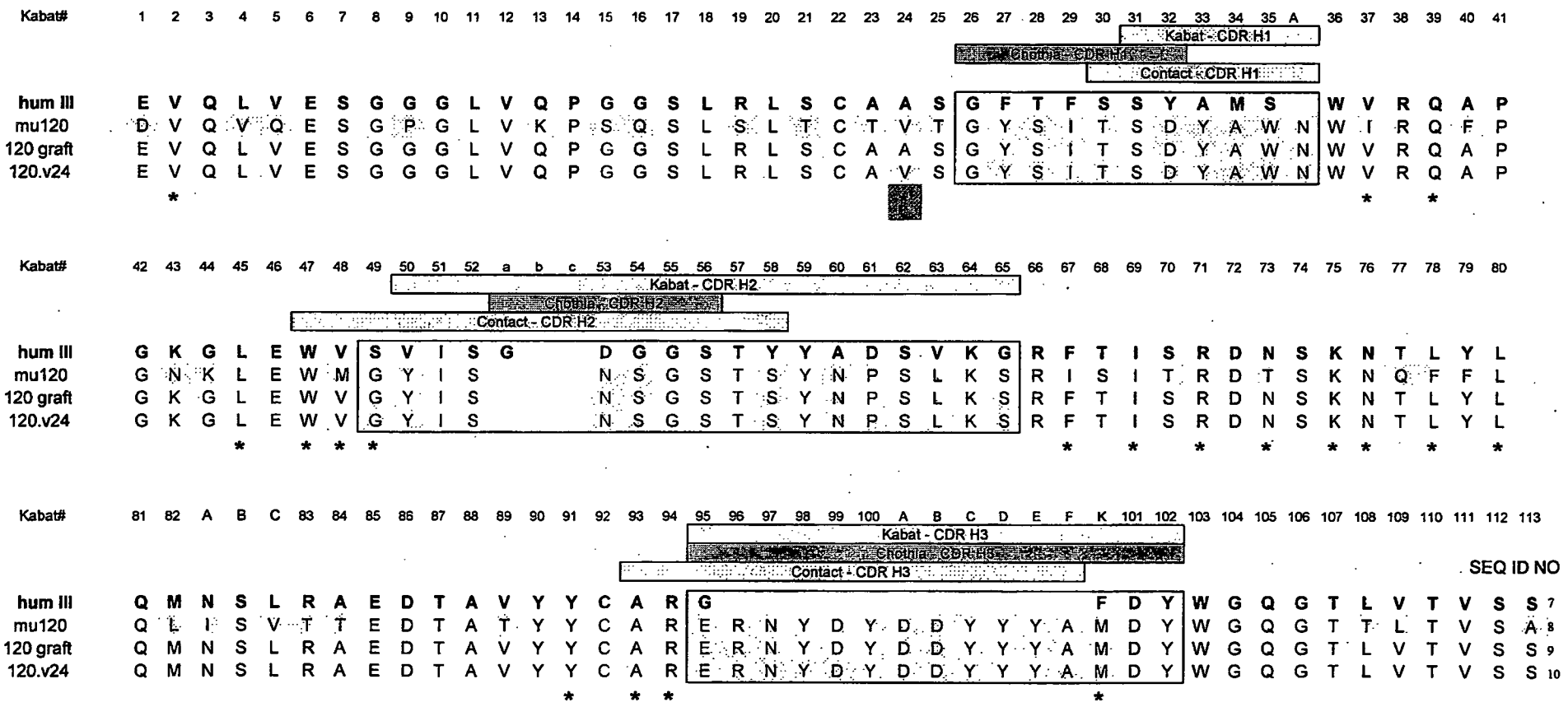


Figure 2B

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I

A Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T -H1- W V R Q A P G Q G L E W M G -H2-

B Q V Q L V Q S G A E V K K P G A S V K V S C K A S -H1- W V R Q A P G Q G L E W M -H2-

C Q V Q L V Q S G A E V K K P G A S V K V S C K A S -H1- W V R Q A P G Q G L E W M -H2-

D Q V Q L V Q S G A E V K K P G A S V K V S C K A S -H1- W V R Q A P G Q G L E W M -H2-

II

A Q V Q L Q E S G P G L V K P S Q T L S L T C T V S G G S V S -H1- W I R Q P P G K G L E W I G -H2-

B Q V Q L Q E S G P G L V K P S Q T L S L T C T V S -H1- W I R Q P P G K G L E W I -H2-

C Q V Q L Q E S G P G L V K P S Q T L S L T C T V S -H1- W I R Q P P G K G L E W I -H2-

D Q V Q L Q E S G P G L V K P S Q T L S L T C T V S -H1- W I R Q P P G K G L E W I -H2-

III

A E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S -H1- W V R Q A P G K G L E W V S -H2-

B E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

C E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

D E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

Acceptor - 1

A E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K -H1- W V R Q A P G K G L E W V S -H2-

B E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

C E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

Acceptor - 2

A E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K -H1- W V R Q A P G K G L E W V S -H2-

B E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

C E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

D E V Q L V E S G G G L V Q P G G S L R L S C A A S -H1- W V R Q A P G K G L E W V -H2-

FIG. 3A

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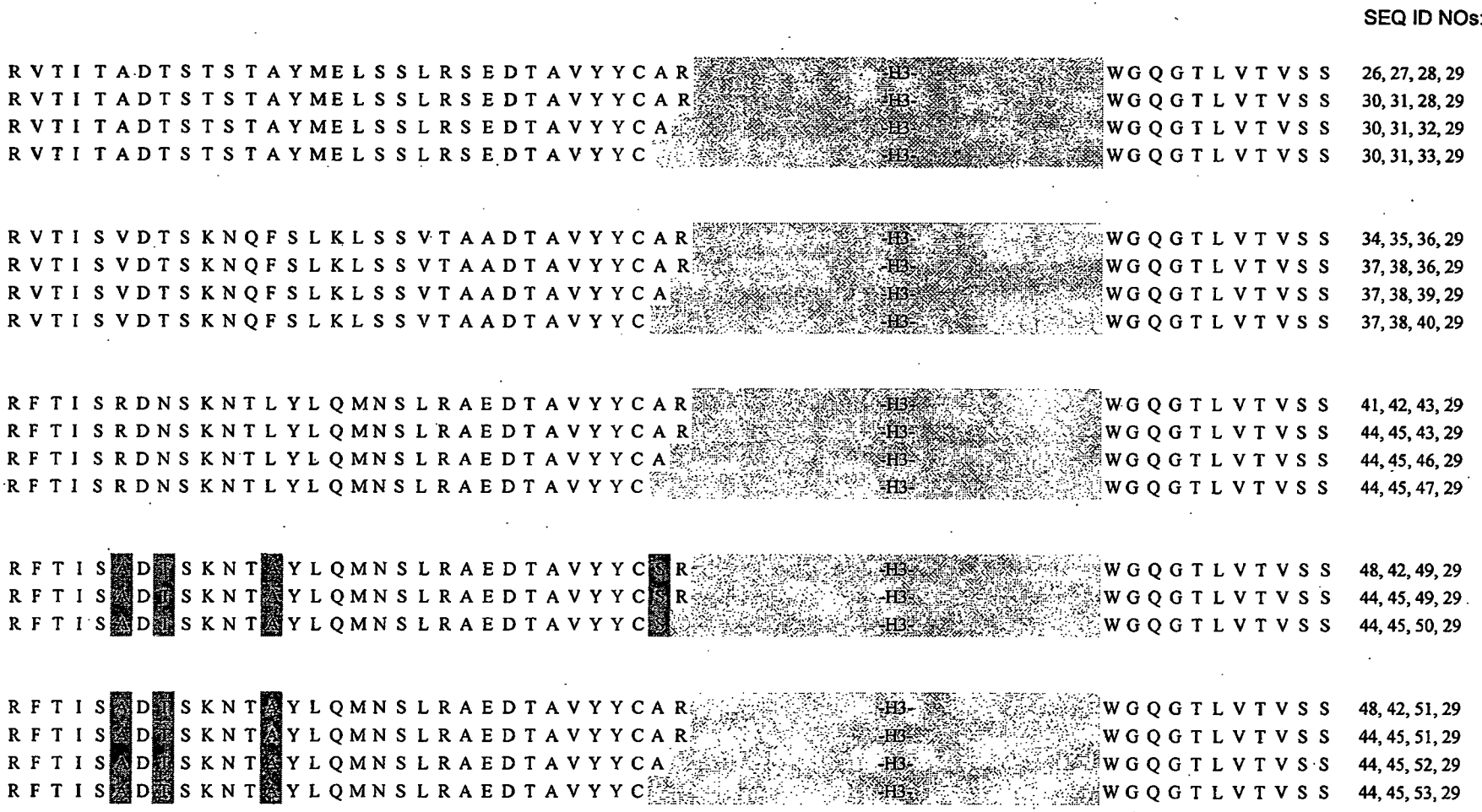


FIG. 3B

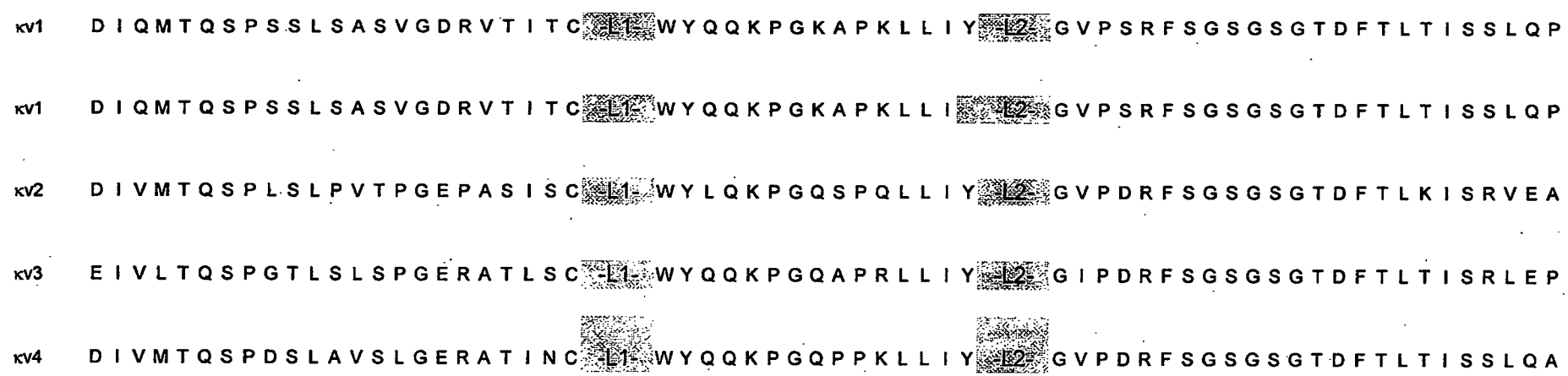


FIG. 4A

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E D F A T Y Y C -L3- F G Q G T K V E I K	SEQ ID NOs:54, 55, 56, 57
E D F A T Y Y C -L3- F G Q G T K V E I K	SEQ ID NOs:54, 58, 56, 57
E D V G V Y Y C -L3- F G Q G T K V E I K	SEQ ID NOs:58, 59, 60, 57
E D F A V Y Y C -L3- F G Q G T K V E I K	SEQ ID NOs:61, 62, 63, 57
E D V A V Y Y C -L3- F G Q G T K V E I K	SEQ ID NOs:64, 65, 66, 57

FIG. 4B

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```
          230        240        250        260        270
humIgG1     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
humIgG2     PAP-PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
humIgG3     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV
humIgG4     PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
               ****                                  *     * *

          280        290        300        310        320
humIgG1     DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG2     DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
humIgG3     DGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG4     DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
                           *    *         *                  *

          330        340        350        360        370
humIgG1     APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
                                          D L
humIgG2     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG3     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG4     SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
            **        *                  *

          380        390        400        410        420
humIgG1     EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG2     EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG3     EWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
humIgG4     EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
               *         *    *              *           *   *

          430        440
humIgG1     EALHNHYTQKSLSLSPGK  (SEQ ID NO:85)
humIgG2     EALHNHYTQKSLSLSPGK  (SEQ ID NO:86)
humIgG3     EALHNRFTQKSLSLSPGK  (SEQ ID NO:87)
humIgG4     EALHNHYTQKSLSLSLGK  (SEQ ID NO:88)
               **         *
```

A Kappa light chain constant region consensus sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:89)

Figure 5

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FACS Analysis / Phage Binding Normalized to Display

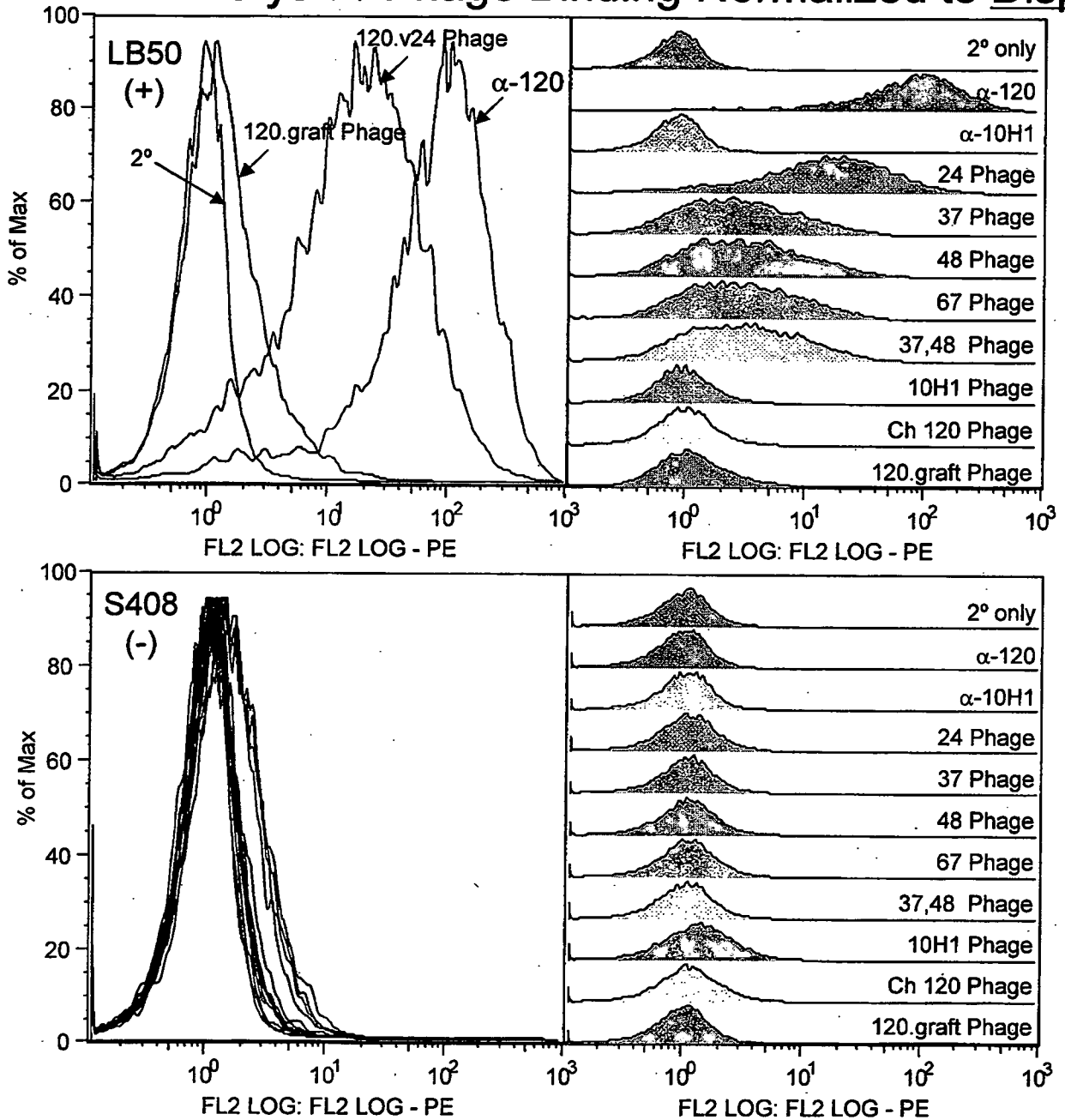


Figure 6

Figure 7A-7C

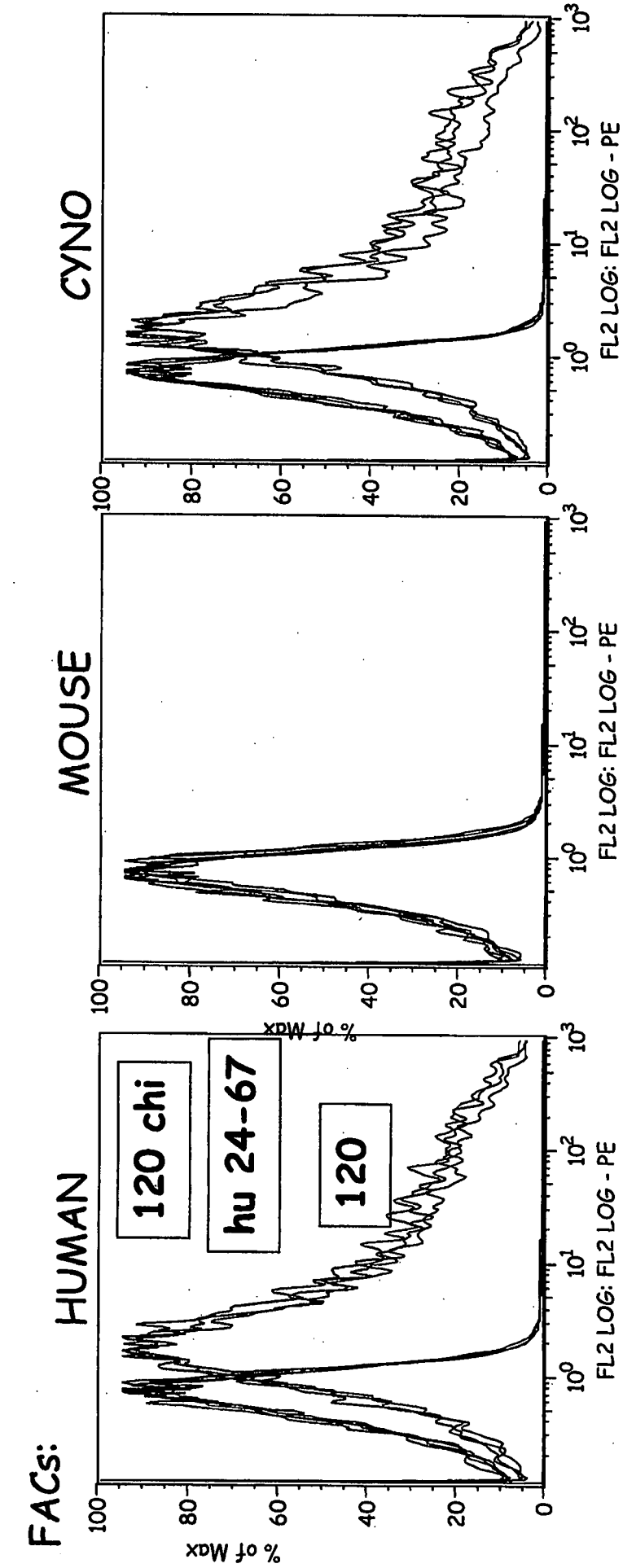


Fig. 7A

Fig. 7B

Fig. 7C

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Figure 7D-7F

FACs:

293 Steap1 LB50

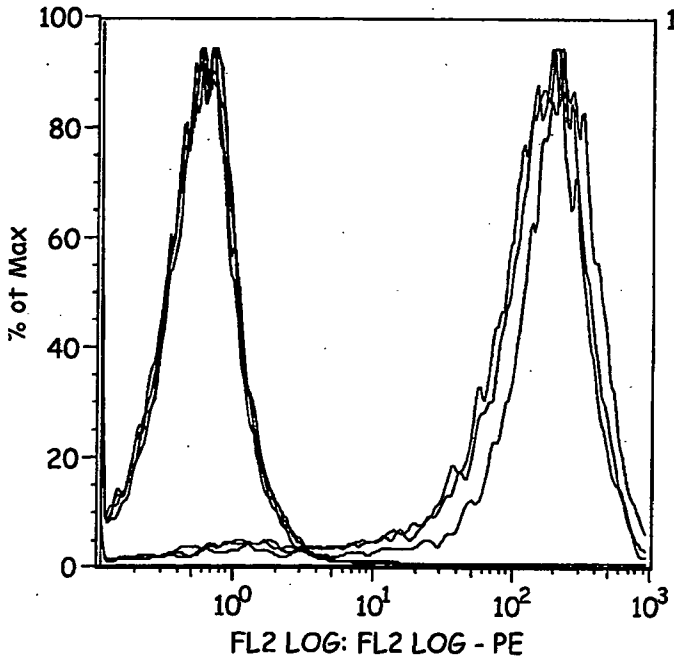


Fig. 7D

PC3 Steap1 PS5.4

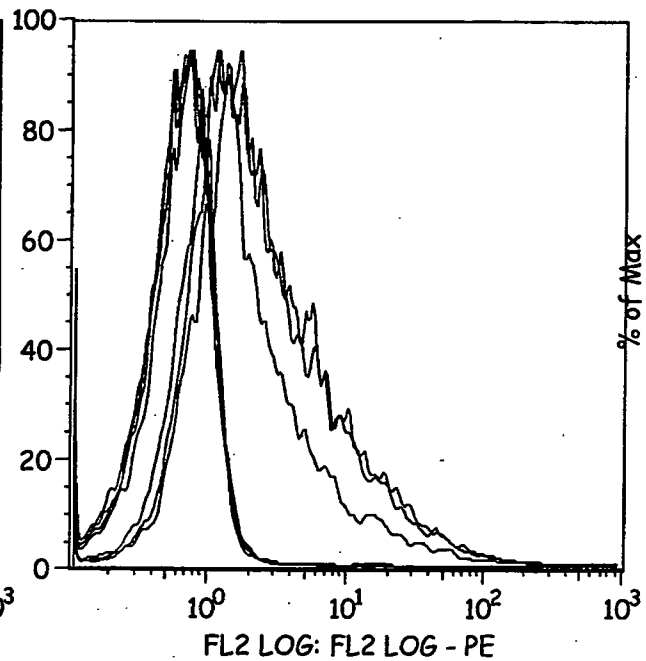


Fig. 7E

LNCAP BR

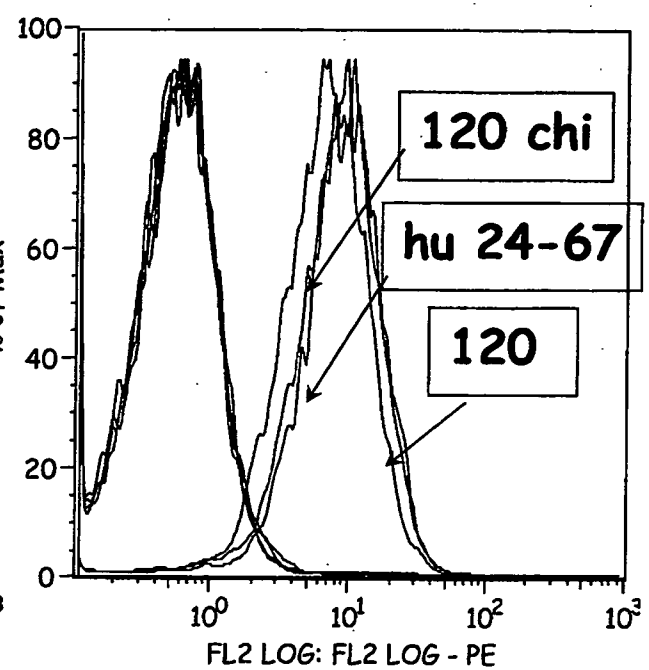
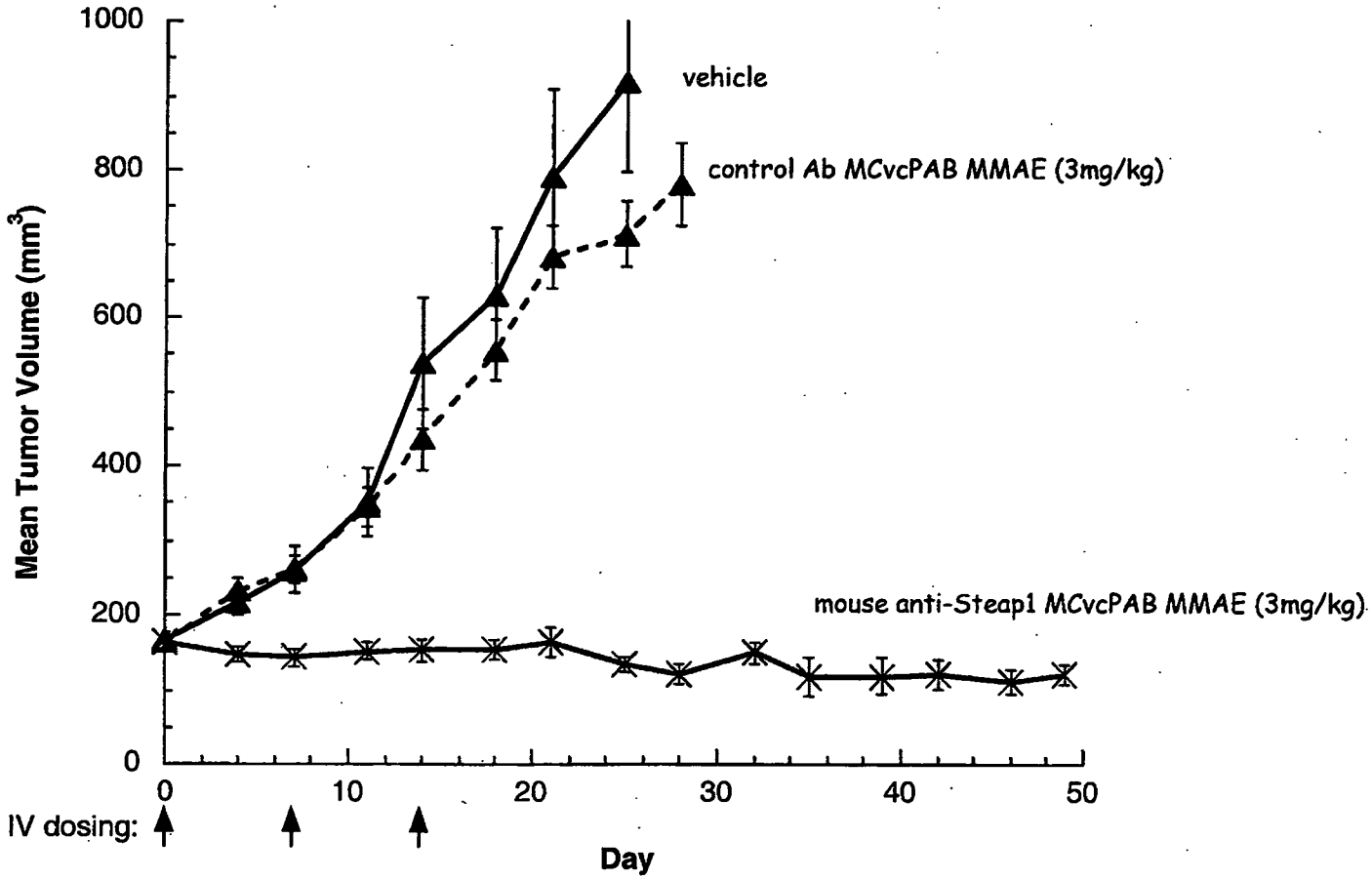


Fig. 7F

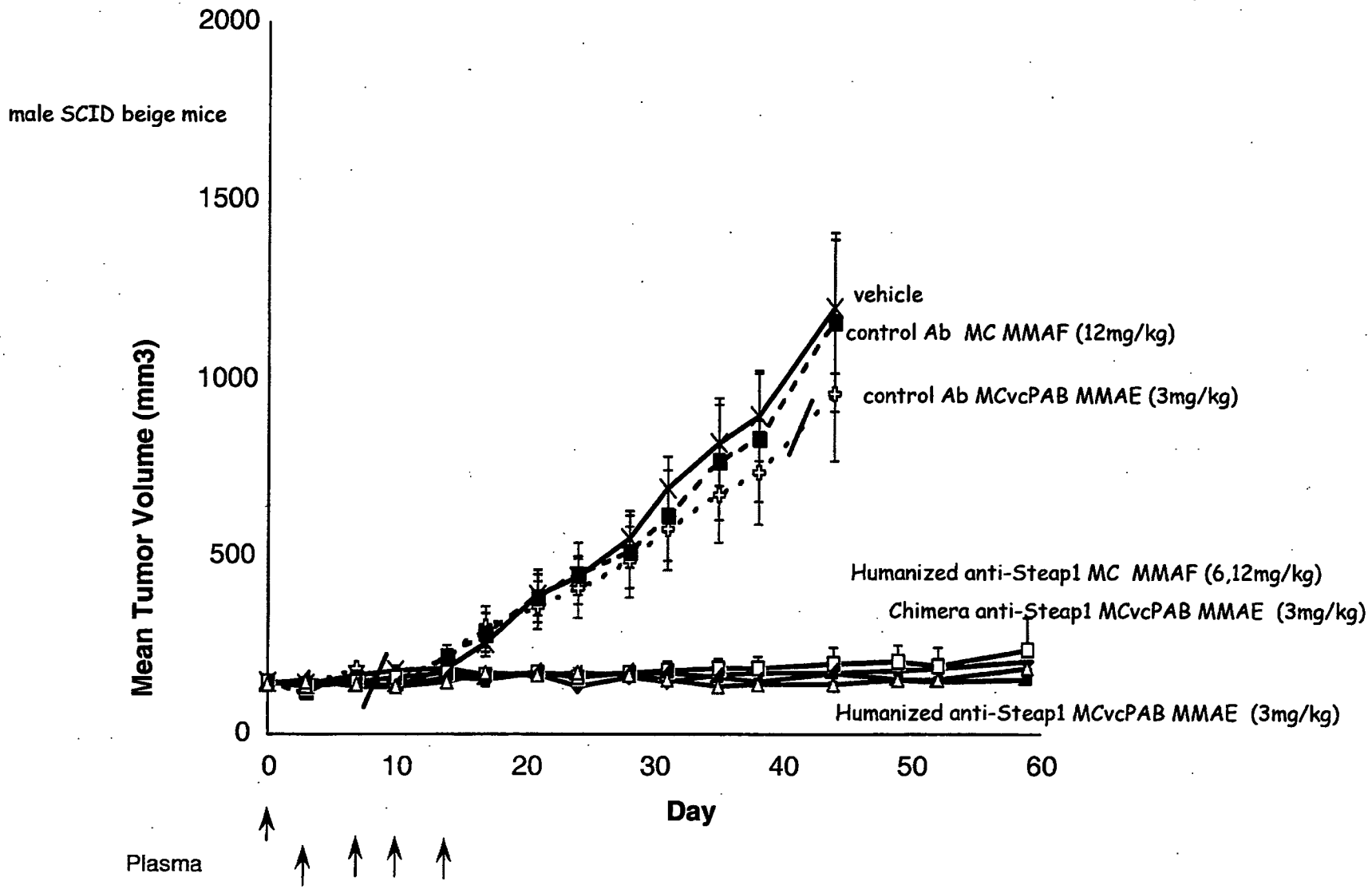
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Figure 8A



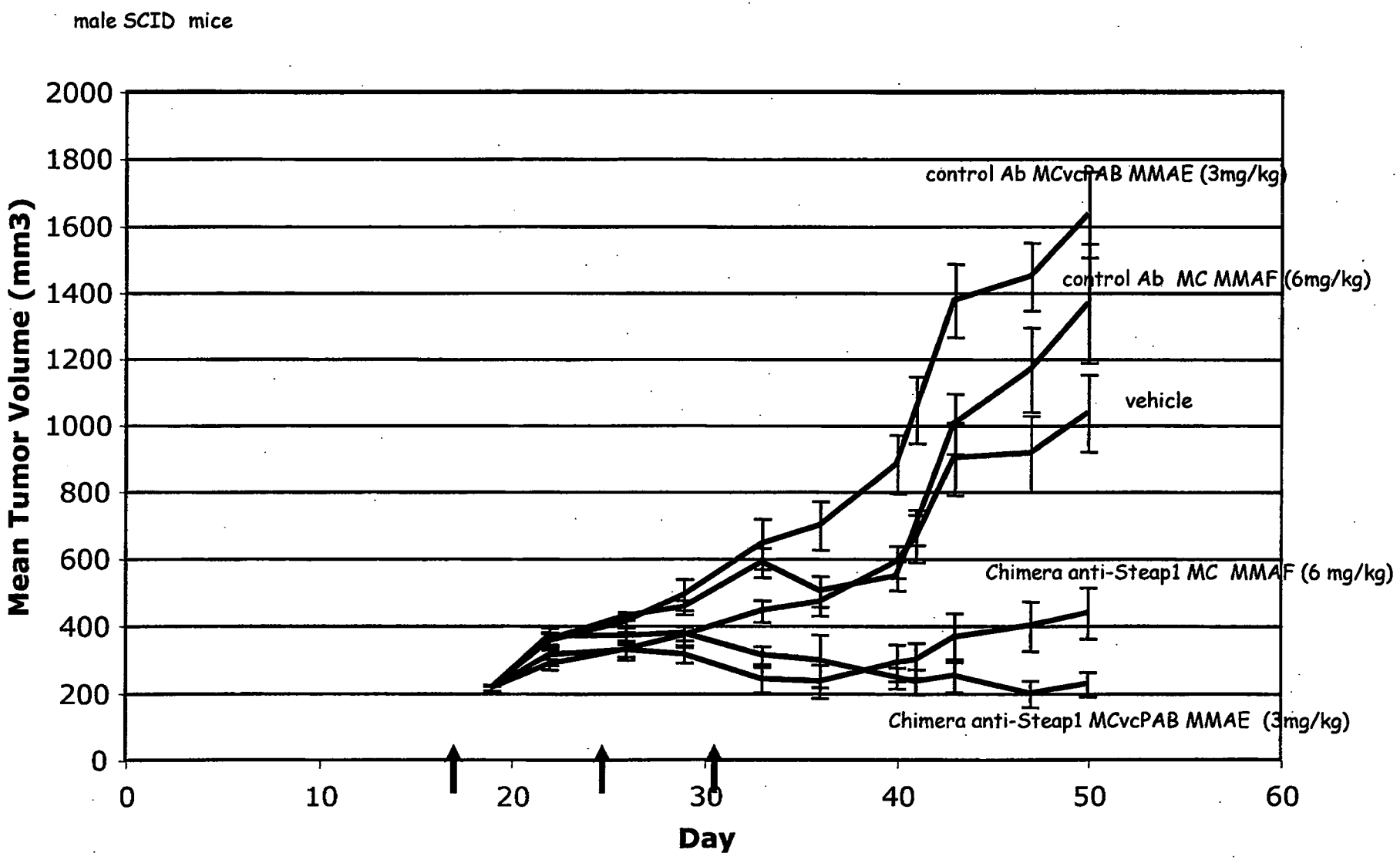
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Figure 8B



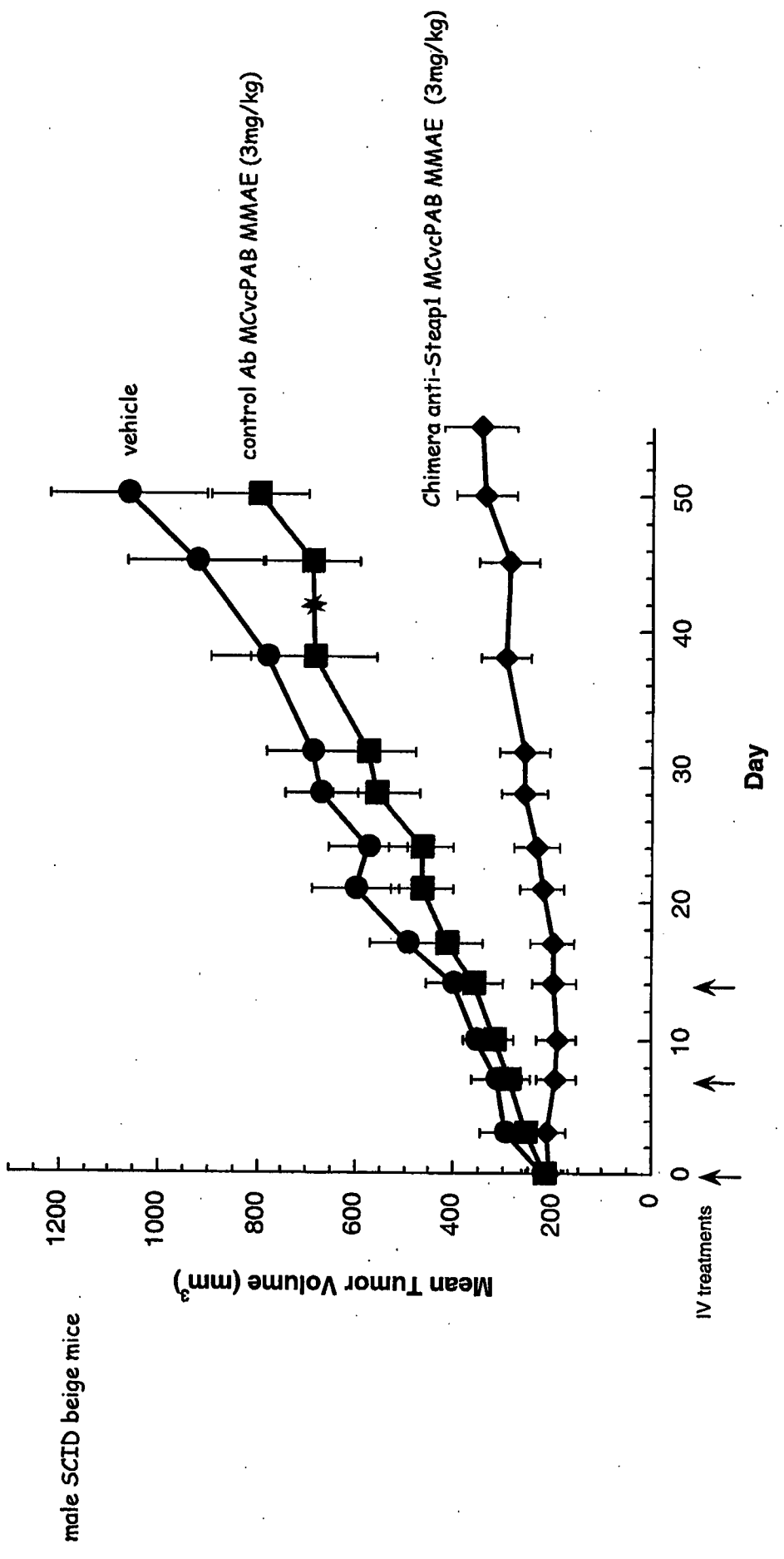
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Figure 9



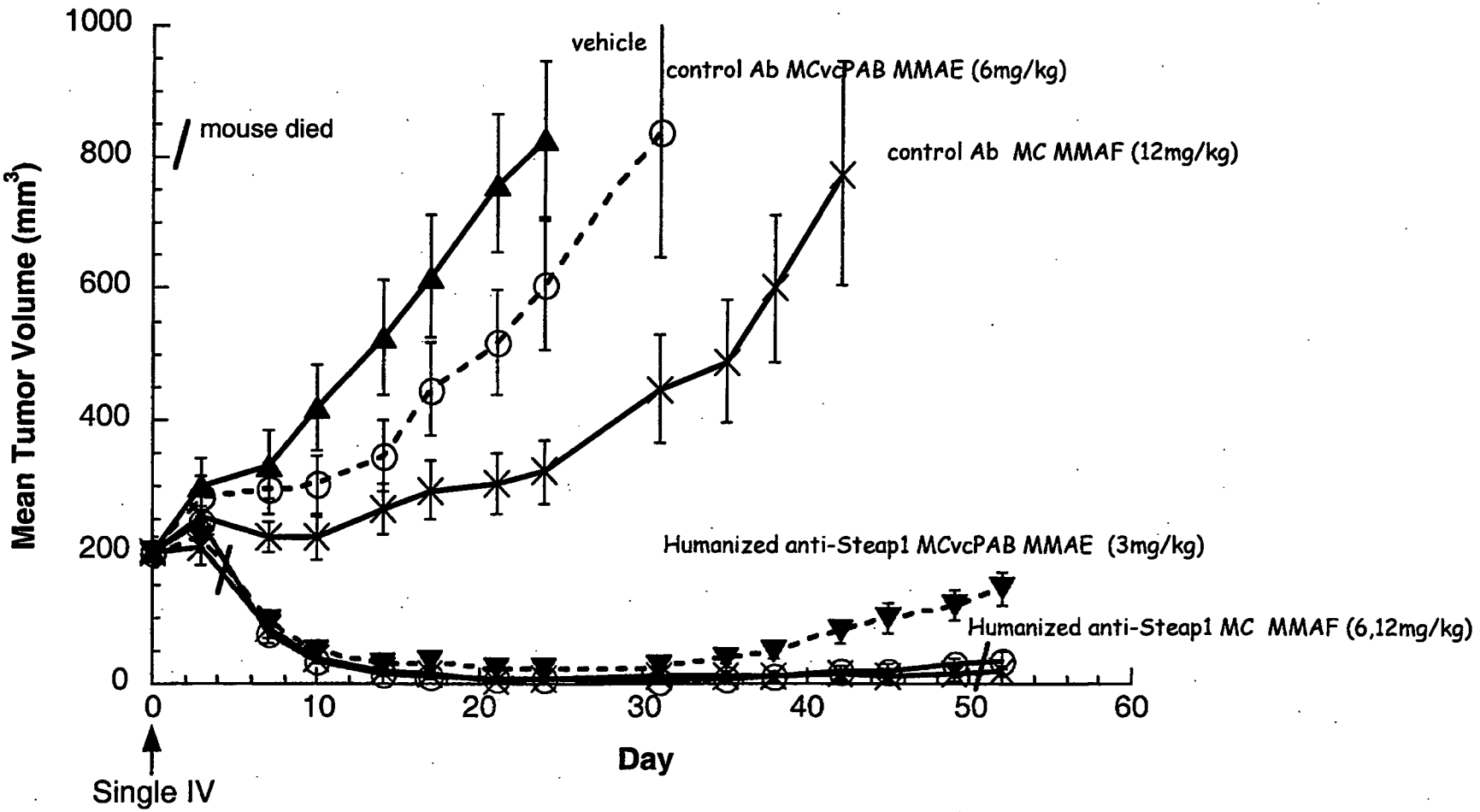
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Figure 10



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Figure 11



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Organizacion y
Digitalizacion CSA LW

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Patente

De Invención

09/34904

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24 de abril de 2009

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NUMERO DE SOLICITUD: *60/921.300*
FECHA DE PRESENTACION: *30 de marzo de 2007*

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60/921300

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SOLICITUD PROVISIONAL PARA CARATULA DE PATENTE

Esta es una solicitud para presentar una SOLICITUD PROVISIONAL bajo 37 CFR 1.53(c)

Etiqueta de Correo Expreso No. EV 766 020 279 US

INVENTOR(ES)

Nombre (primer y segundo [si hubiese])	Apellido	Residencia (Ciudad y Estado o País Extranjero)
Mark S.	Dennis	South San Francisco, CA
Bonnee	Rubinfeld	South San Francisco, CA
Paul	Polakis	South San Francisco, CA
Aya	Jakobovits	Santa Monica, CA

☐ Se nombran inventores adicionales en las hojas ____ numeradas separadamente adjuntas al presente

TITULO DE LA INVENCION (500 caracteres max)

ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

DIRECCION DE CORRESPONDENCIA

Dirigir toda la correspondencia a:

Coloque el Número del Código de Barras Aquí

Cliente

☒ Número del Cliente **25213** Número del Cliente Aquí

O

☐ Nombre de la Firma o Persona Natural	GINGER R. DREGER, ESQ., HELLER EHRMAN LLP				
Dirección	275 Middlefield Road				
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País	EU	Teléfono	650.324.7000	Fax	650.324.0638

ADJUNTE PARTES DE LA SOLICITUD (marque todas las que aplican)

☒ Especificación (Número de Páginas) 183 páginas ☐ CD(s), Número

☒ Dibujos (Número de Páginas) 29 páginas

☐ Hoja de Datos de la Solicitud. Ver 37 CFR 1.76 ☐ Otros (especifique) Postal

METODO DE PAGO (marque uno)

☒ El solicitante reivindica estado de entidad pequeña. Ver 37 CFR 1.27 ☐ Se adjunta un cheque o giro postal para cubrir derechos de presentación Provisional ☒ Por el presente el Comisionado está autorizado para cobrar derechos de presentación y acreditar a la Cuenta de Depósito Número 08-1641 (Ref. Expediente No. 39766-0160 PR6 ☒ Por favor cargue a la Cuenta de Depósito Número 08-1641 para cubrir derechos adicionales que se venzan o acreditar sobrepago a esa cuenta.	SUMA DERECHOS PRESENTACION PROVISIONAL $100.00

[redacted]a invención fue hecha por una agencia del gobierno de los Estados Unidos o bajo un contrato con una agencia del gobierno de los Estados Unidos.

☒ No

☐ Sí, el nombre de la agencia del Gobierno de los EE.UU. y el número del contrato del Gobierno son: _____.

Presentado respetuosamente,

FIRMA (ilegible) Fecha: 30 de marzo de 2007

NOMBRE IMPRESO: Barrie D. Greene, Ph.d REGISTRO NO. 46.740

TELEFONO (650) 324-7000 NÚMERO EXPEDIENTE: 39766-0160 PT6

USAR UNICAMENTE PARA PRESENTAR UNA SOLICITUD PROVISIONAL DE PATENTE

[redacted]

Traducido según mi mejor saber y entender, este día 12 de mayo de 2009, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTORA OFICIAL
RES. 2177/89 MINJUSTICIA

ANITA ESCOBAR DE TAMAYO

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THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 24, 2009

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/921,300*
FILING DATE: *March 30, 2007*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/921,300*

By Authority of the
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M. Tarver

M. TARVER
Certifying Officer

U.S. PTO
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

Express Mail Label No. EV 766 020 279 US

INVENTOR(s)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Mark S.	**Dennis**	**South San Francisco, CA**
Bonnee	**Rubinfeld**	**South San Francisco, CA**
Paul	**Polakis**	**South San Francisco, CA**
Aya	**Jakobovits**	**Santa Monica, CA**

☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 Characters Max)

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

CORRESPONDENCE ADDRESS

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Country	**USA**	*Telephone*	**650.324.7000**	*FAX*	**650.324.0638**

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification (Number of Pages) **183 pages** ☐ CD(s), Number

☒ Figures (Number of Pages) ***29 sheets***

☐ Application Data Sheet. See 37 CFR 1.76 ☒ Other (specify) **Postcard**

METHOD OF PAYMENT (check one)

☒ **Applicant claims small entity status. See 37 CFR 1.27.**

☐ A check or money order is enclosed to cover the Provisional filing fees

☒ **The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 08-1641 (Ref. Atty. Docket No. 39766-0160 PR6**

☒ **Please charge Deposit Account No. 08-1641 to cover any additional fees that may be due or credit any overpayment to that account.**

PROVISIONAL FILING FEE AMOUNT

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ____

Respectfully submitted,

SIGNATURE: _____

TYPE OR PRINTED NAME: Barrie D. Greene, Ph.d

TELEPHONE: (650) 324-7000

Date: **MARCH 30, 2007**

REGISTRATION NO. 46,740

DOCKET NUMBER **39766-0160 PR6**

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and subjecting the complete provisional application to the PTO. 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, Washington, D.C 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

FIELD OF THE INVENTION

The present invention relates to anti-STEAP-1 antibodies and immunconjugates thereof. The invention further relates to methods of using anti-STEAP-1 antibodies and immunconjugates thereof.

BACKGROUND

In humans, prostate cancer is one of the most commonly diagnosed malignancies in males and is the second leading cause of cancer related death in men. The American Cancer Society estimates that for the year 2000, 180,400 new cases of prostate cancer will be diagnosed with 31,900 deaths from the disease. In advanced stages, prostate cancer metastasizes to the bone. While advances in early diagnosis and treatment of locally confined tumors have been achieved, prostate cancer is incurable once it has metastasized. Patients with metastatic prostate cancer on hormonal therapy will eventually develop an androgen-refractory (androgen independent) state that will lead to disease progression and death. Currently, prostate-specific antigen (PSA) is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer. However, widespread use of PSA as a tool for screening is controversial since PSA fails to discriminate accurately between benign and malignant prostate disease.

Depending on the stage of the cancer, prostate and bladder cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, chemotherapy, androgen deprivation (e.g., hormonal therapy) in the case of prostate cancer. While surgical or radiation therapy significantly improves survival in patients with early stages of the disease, the therapeutic options are very limited for advanced cases, particularly for tumor recurrences following hormone ablation. The majority of patients who undergo hormone therapy progress to develop androgen-independent disease. Currently, there is no effective treatment for the 20-40% of prostate cancer patients who develop recurrent disease after surgery or radiation therapy, or for those in whom the cancer has metastasized at the time of diagnosis. Chemotherapy has its toxic side effects, especially in elderly patients. Development of new forms of therapy especially for disease refractory to androgen deprivation is an urgent need in the management of prostatic carcinoma.

The identification of a novel cell surface antigen, STEAP-1 has been described (see US6,329,503). STEAP-1 is member of cell surface serpentine transmembrane antigens. It is expressed predominantly in the prostate cancer, and thus members of this family have been tered "STEAP" (Six Transmembrane Epithelial Antigens of the Prostate). Human STEAP proteins exhibit a high degree of structural

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conservation within the family by show no significant structural homology to any known human proteins. STEAP-1 appears to be a type IIIa membrane protein expressed predominantly in prostate cells in normal human tissues. Structurally, STEAP-1 is a 339 amino acid protein characterized by a molecular topology of six transmembrane domains and intracellular N- and C-termini, suggesting that it folds in a "serpentine" manner into three extracellular and two intracellular loops. STEAP-1 protein expression is maintained at high levels across various states of prostate cancer. STEAP-1 is highly over-expressed in other human cancers such as lung and colon. Murine antibodies have been raised to human STEAP-1 fragments and the antibodies were shown to bind STEAP-1 on the cell surface (see US20040253232A1).

Antibody-based therapy has proved very effective in the treatment of various cancers. For example, HERCEPTIN® and RITUXAN® (both from Genentech, S. San Francisco), have been used successfully to treat breast cancer and non-Hodgkin's lymphoma, respectively. HERCEPTIN® is a recombinant DNA-derived humanized monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) proto-oncogene. HER2 protein overexpression is observed in 25-30% of primary breast cancers. RITUXAN® is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. Both these antibodies are produced in CHO cells.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4975278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., Cancer Immunol. Immunother. 21:183-87 (1986)). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Kerr et al (1997) Bioconjugate Chem. 8(6):781-784; Mandler et al (2000) Journal of the 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).

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The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition (Meyer, D.L. and Senter, P.D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" in Annual Reports in Medicinal Chemistry, Vol 38 (2003) Chapter 23, 229-237). Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is being developed for the treatment of cancers that express CanAg antigen, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The same maytansinoid drug moiety, DM1, was linked through a non-disulfide linker, SMCC, to a mouse murine monoclonal antibody, TA.1 (Chari et al. (1992) Cancer Research 52:127-131). This conjugate was reported to be 200-fold less potent than the corresponding disulfide linker conjugate. The SMCC linker was considered therein to be "noncleavable."

Several short peptidic compounds have been isolated from the marine mollusk, Dolabella auricularia, and found to have biological activity (Pettit et al (1993) Tetrahedron 49:9151; Nakamura et al (1995) Tetrahedron Letters 36:5059-5062; Sone et al (1995) Journal Org Chem. 60:4474). Analogs of these compounds have also been prepared, and some were found to have biological activity (for a review, see Pettit et al (1998) Anti-Cancer Drug Design 13:243-277). For example, auristatin E (US 5635483) is a synthetic analogue of the marine natural product Dolastatin 10, an agent that inhibits tubulin polymerization by binding to the same site on tubulin as the anticancer drug vincristine (G. R. Pettit, (1997) Prog. Chem. Org. Nat. Prod. 70:1-79). Dolastatin 10, auristatin PE, and auristatin E are linear

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peptides having four amino acids, three of which are unique to the dolastatin class of compounds, and a C-terminal amide.

The auristatin peptides, auristain E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to: (i) chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas); (ii) cAC10 which is specific to CD30 on hematological malignancies (Klussman, et al (2004), Bioconjugate Chemistry 15(4):765-773; Doronina et al (2003) Nature Biotechnology 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco et al (2003) Blood 102(4):1458-1465; US 2004/0018194; (iii) anti-CD20 antibodies such as Rituxan® (rituximab) (WO 04/032828) for the treatment of CD20-expressing cancers and immune disorders; (iv) anti-EphB2 antibodies 2H9 and anti-IL-8 for treatment of colorectal cancer (Mao, et al (2004) Cancer Research 64(3):781-788); (v) E-selectin antibody (Bhaskar et al (2003) Cancer Res. 63:6387-6394); and (vi) other anti-CD30 antibodies (WO 03/043583). Monomethylauristatin (MMAE) has also been conjugated to 2H9, an antibody against EphB2R which is a type 1 TM tyrosine kinase receptor with close homology between mouse and human, and is over-expressed in colorectal cancer cells (Mao et al (2004) Cancer Res. 64:781-788).

Monomethylauristatin MMAF, a variant of auristatin E (MMAE) with a phenylalanine at the C-terminus (US 5767237; US 6124431), has been reported to be less potent than MMAE, but more potent when conjugated to monoclonal antibodies (Senter et al, Proceedings fo the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004). Auristatin F phenylene diamine (AFP); a phenylalanine variant of MMAE was linked to an anti-CD70 mAb, 1F6, through the C-terminus of 1F6 via a phenylene diamine spacer (Law et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 625, presented March 28, 2004).

There exists a need in the art for additional drugs to treat various cancers such as cancers and metatases of cancers in the prostate, lung and colon. Particularly useful drugs for this purpose include prostate, lung or colon cell targeted anti-STEAP-1 antibody-drug conjugates having a significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

The recitation of any reference in this application is not an admission that the reference is prior art to this application. All references cited herein, including patents, patent applications and publications, are incorporated by reference in their entirety.

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SUMMARY

The invention provides anti-STEAP-1 antibodies and methods of using the same.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) or a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9). In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence shown in Figure 2A (SEQ ID NO:6) and a heavy chain variable domain comprising the amino acid sequence shown in Figure 2B (SEQ ID NO:9).

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79.

In one aspect, the antibody comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In one aspect, an antibody that binds to STEAP-1 is provided, wherein the antibody comprises a heavy chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NOs:9 or 10. In one embodiment, the antibody comprises a heavy chain variable domain framework region 1 of SEQ ID NO:25 or a heavy chain variable domain framework region 2 of SEQ ID NO:75 or 76 or 77 or a heavy chain variable domain framework region 3 of SEQ ID NO:78 or 79. In an embodiment, the antibody further comprises a light chain variable domain having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to an amino acid sequence of SEQ ID NO:6. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:6.

In certain embodiments, a polynucleotide encoding any of the above antibodies is provided. In one embodiment, a vector comprising the polynucleotide is provided. In one embodiment, a host cell comprising the vector is provided. In one embodiment, the host cell is eukaryotic. In one embodiment, the host cell is a Chinese hamster ovary (CHO) cell. In one embodiment, a method of making an anti-STEAP-

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1 antibody is provided, wherein the method comprises culturing the host cell under conditions suitable for expression of the polynucleotide encoding the antibody, and isolating the antibody.

In one aspect, an antibody that binds to STEAP-1 expressed on the surface of a cell is provided. In one embodiment, the antibody binds to an epitope within a region of human or mouse STEAP-1. In one embodiment, the cell is mammalian cell. In one embodiment, the cell is a human cell. In one embodiment, the cell is a cancer cell. In one embodiment the cell is a prostate, lung or colon cell. In one embodiment the cancer cell is a prostate cancer cell. In another embodiment, the cell cell from a metastisis of a primary prostate, lung or colon cancer.

In certain embodiments, any of the above antibodies is a monoclonal antibody. In one embodiment, the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one embodiment, the antibody is humanized. In one embodiment, the antibody is human.

In one aspect, a method of detecting the presence of STEAP-1 in a biological sample is provided, the method comprising contacting the biological sample with any of the above antibodies under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1. In one embodiment, the biological sample comprises prostate cells. In one embodiment, the biological sample is from a mammal experiencing or suspected of experiencing a prostate cell disorder and/or a cell proliferative disorder of cells or tissues including, but not limited to, prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma as well as metastases of primary prostate, lung, colon, bladder, and ovarian cancers and Ewing's sarcoma. See, for example, (see US6,329,503; and Rodeberg, D.A. et al., Clin. Cancer Res. 11(12):4545-4552 (2005)).

In one aspect, a method of diagnosing a cell proliferative disorder associated with increased expression of STEAP-1 is provided, the method comprising contacting a test cell with any of the above antibodies; determining the level of expression of STEAP-1 by detecting binding of the antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell, wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a cell proliferative disorder associated with increased expression of STEAP-1. In one embodiment, the test cell is a cell from a patient suspected of having a cell proliferative disorder, such as a prostate proliferative disorder. In one embodiment, the cell proliferative disorder is selected from prostate cell disorders including but not limited to prostate cancer. In one embodiment, the method comprises determining the level of expression of STEAP-1 on the surface of the test cell (such as, for example, a prostate cancer cell) and comparing the level of expression of STEAP-1 on the surface of the test cell with the level of expression of STEAP-1 on the surface of the control cell (such, for example, as a normal prostate cell other than an abnormally proliferating prostate cell).

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In one aspect, a method of diagnosing a cell proliferative disorder associated with an increase in cells, such as prostate cells, expressing STEAP-1 is provided, the method comprising contacting a test cells in a biological sample with any of the above antibodies; determining the level of antibody bound to test cells in the sample by detecting binding of the antibody to STEAP-1; and comparing the level of antibody bound to cells in a control sample, wherein the level of antibody bound is normalized to the number of STEAP-1-expressing cells in the test and control samples, and wherein a higher level of antibody bound in the test sample as compared to the control sample indicates the presence of a cell proliferative disorder associated with cells expressing STEAP-1.

In one aspect, a method of detecting soluble STEAP-1 in blood or serum, the method comprising contacting a test sample of blood or serum from a mammal suspected of experiencing a prostate cell proliferative disorder with an anti-STEAP-1 antibody of the invention and detecting a increase in soluble STEAP-1 in the test sample relative to a control sample of blood or serum from a normal mammal. In an embodiment, the method of detecting is useful as a method of diagnosing a prostate cell proliferative disorder associated with an increase in soluble STEAP-1 in blood or serum of a mammal.

In one aspect, the antibodies of the invention include cysteine engineered antibodies where one or more amino acids of a parent antibody are replaced with a free cysteine amino acid as disclosed in WO2006/034488 (herein incorporated by reference in its entirety). A cysteine engineered antibody comprises one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0. A free cysteine amino acid is a cysteine residue which has been engineered into the parent antibody and is not part of a disulfide bridge. Cysteine engineered antibodies are useful for attachment of cytotoxic and/or imaging compounds at the site of the engineered cysteine through, for example, a maleimide or haloacetyl. The nucleophilic reactivity of the thiol functionality of a Cys residue to a maleimide group is about 1000 times higher compared to any other amino acid functionality in a protein, such as amino group of lysine residues or the N-terminal amino group. Thiol specific functionality in iodoacetyl and maleimide reagents may react with amine groups, but higher pH (>9.0) and longer reaction times are required (Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London).

Cysteine engineered antibodies may be useful in the treatment of cancer and include antibodies specific for cell surface and transmembrane receptors, and tumor-associated antigens (TAA). Such antibodies may be used as naked antibodies (unconjugated to a drug or label moiety) or as antibody-drug conjugates (ADC). Cysteine engineered antibodies of the invention may be site-specifically and efficiently coupled with a thiol-reactive reagent. The thiol-reactive reagent may be a multifunctional linker reagent, a capture label reagent, a fluorophore reagent, or a drug-linker intermediate. The cysteine engineered antibody may be labeled with a detectable label, immobilized on a solid phase support and/or conjugated with a drug moiety. Thiol reactivity may be generalized to any antibody where substitution of amino acids

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with reactive cysteine amino acids may be made within the ranges in the light chain selected from amino acid ranges: L-10 to L-20; L-38 to L-48; L-105 to L-115; L-139 to L-149; L-163 to L-173; and within the ranges in the heavy chain selected from amino acid ranges: H-35 to H-45; H-83 to H-93; H-114 to H-127; and H-170 to H-184, and in the Fc region within the ranges selected from H-268 to H-291; H-319 to H-344; H-370 to H-380; and H-395 to H-405, where the numbering of amino acid positions begins at position 1 of the Kabat numbering system (Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and continues sequentially thereafter as disclosed in WO2006034488. Thiol reactivity may also be generalized to certain domains of an antibody, such as the light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. Cysteine replacements resulting in thiol reactivity values of 0.6 and higher may be made in the heavy chain constant domains α, δ, ϵ, γ, and μ of intact antibodies: IgA, IgD, IgE, IgG, and IgM, respectively, including the IgG subclasses: IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. Such antibodies and their uses are disclosed in WO2006/034488.

Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, transmembrane proteins, signalling proteins, cell survival regulatory factors, cell proliferation regulatory factors, molecules associated with (for e.g., known or suspected to contribute functionally to) tissue development or differentiation, lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in vasculogenesis and molecules associated with (for e.g., known or suspected to contribute functionally to) angiogenesis.

An antibody of the invention may be conjugated to other thiol-reactive agents in which the reactive group is, for example, a maleimide, an iodoacetamide, a pyridyl disulfide, or other thiol-reactive conjugation partner (Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Hermanson, G. in Bioconjugate Techniques (1996) Academic Press, San Diego, pp. 40-55, 643-671). The partner may be a cytotoxic agent (e.g. a toxin such as doxorubicin or pertussis toxin), a fluorophore such as a fluorescent dye like fluorescein or rhodamine, a chelating agent for an imaging or radiotherapeutic metal, a peptidyl or non-peptidyl label or detection tag, or a clearance-modifying agent such as various isomers of polyethylene glycol, a peptide that binds to a third component, or another carbohydrate or lipophilic agent.

In one aspect, antibodies of the invention may be conjugated with any label moiety which can be covalently attached to the antibody through a reactive moiety, an activated moiety, or a reactive cysteine

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thiol group (Singh et al (2002) Anal. Biochem. 304:147-15; Harlow E. and Lane, D. (1999) Using Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press, Cold Spring Harbor, NY; Lundblad R.L. (1991) Chemical Reagents for Protein Modification, 2nd ed. CRC Press, Boca Raton, FL). The attached label may function to: (i) provide a detectable signal; (ii) interact with a second label to modify the detectable signal provided by the first or second label, e.g. to give FRET (fluorescence resonance energy transfer); (iii) stabilize interactions or increase affinity of binding, with antigen or ligand; (iv) affect mobility, e.g. electrophoretic mobility or cell-permeability, by charge, hydrophobicity, shape, or other physical parameters, or (v) provide a capture moiety, to modulate ligand affinity, antibody/antigen binding, or ionic complexation.

Labelled cysteine engineered antibodies may be useful in diagnostic assays, e.g., for detecting expression of an antigen of interest in specific cells, tissues, or serum. For diagnostic applications, the antibody will typically be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

Radioisotopes (radionuclides), such as ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, or ^{213}Bi. Radioisotope labelled antibodies are useful in receptor targeted imaging experiments. The antibody can be labeled with ligand reagents that bind, chelate or otherwise complex a radioisotope metal where the reagent is reactive with the engineered cysteine thiol of the antibody, using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al, Ed. Wiley-Interscience, New York, NY, Pubs. (1991). Chelating ligands which may complex a metal ion include DOTA, DOTP, DOTMA, DTPA and TETA (Macrocyclics, Dallas, TX). Radionuclides can be targetted via complexation with the antibody-drug conjugates of the invention (Wu et al (2005) Nature Biotechnology 23(9):1137-1146).

Linker reagents such as DOTA-maleimide (4-maleimidobutyramidobenzyl-DOTA) can be prepared by the reaction of aminobenzyl-DOTA with 4-maleimidobutyric acid (Fluka) activated with isopropylchloroformate (Aldrich), following the procedure of Axworthy et al (2000) Proc. Natl. Acad. Sci. USA 97(4):1802-1807). DOTA-maleimide reagents react with the free cysteine amino acids of the cysteine engineered antibodies and provide a metal complexing ligand on the antibody (Lewis et al (1998) Bioconj. Chem. 9:72-86). Chelating linker labelling reagents such as DOTA-NHS (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono (N-hydroxysuccinimide ester) are commercially available (Macrocyclics, Dallas, TX). Receptor target imaging with radionuclide labelled antibodies can provide a marker of pathway activation by detection and quantitation of progressive accumulation of antibodies in tumor tissue (Albert et al (1998) Bioorg. Med. Chem. Lett. 8:1207-1210). The conjugated radio-metals may remain intracellular following lysosomal degradation.

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Metal-chelate complexes suitable as antibody labels for imaging experiments are disclosed: US 5342606; US 5428155; US 5316757; US 5480990; US 5462725; US 5428139; US 5385893; US 5739294; US 5750660; US 5834456; Hnatowich et al (1983) J. Immunol. Methods 65:147-157; Meares et al (1984) Anal. Biochem. 142:68-78; Mirzadeh et al (1990) Bioconjugate Chem. 1:59-65; Meares et al (1990) J. Cancer1990, Suppl. 10:21-26; Izard et al (1992) Bioconjugate Chem. 3:346-350; Nikula et al (1995) Nucl. Med. Biol. 22:387-90; Camera et al (1993) Nucl. Med. Biol. 20:955-62; Kukis et al (1998) J. Nucl. Med. 39:2105-2110; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Camera et al (1994) J. Nucl. Med. 21:640-646; Ruegg et al (1990) Cancer Res. 50:4221-4226; Verel et al (2003) J. Nucl. Med. 44:1663-1670; Lee et al (2001) Cancer Res. 61:4474-4482; Mitchell, et al (2003) J. Nucl. Med. 44:1105-1112; Kobayashi et al (1999) Bioconjugate Chem. 10:103-111; Miederer et al (2004) J. Nucl. Med. 45:129-137; DeNardo et al (1998) Clinical Cancer Research 4:2483-90; Blend et al (2003) Cancer Biotherapy & Radiopharmaceuticals 18:355-363; Nikula et al (1999) J. Nucl. Med. 40:166-76; Kobayashi et al (1998) J. Nucl. Med. 39:829-36; Mardirossian et al (1993) Nucl. Med. Biol. 20:65-74; Roselli et al (1999) Cancer Biotherapy & Radiopharmaceuticals, 14:209-20.

(b) Fluorescent labels such as rare earth chelates (europium chelates), fluorescein types including FITC, 5-carboxyfluorescein, 6-carboxy fluorescein; rhodamine types including TAMRA; dansyl; Lissamine; cyanines; phycoerythrins; Texas Red; and analogs thereof. The fluorescent labels can be conjugated to antibodies using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescent dyes and fluorescent label reagents include those which are commercially available from Invitrogen/Molecular Probes (Eugene, OR) and Pierce Biotechnology, Inc. (Rockford, IL).

(c) Various enzyme-substrate labels are available or disclosed (US 4275149). The enzyme generally catalyzes a chemical alteration of a chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; US 4737456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al (1981) "Methods

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for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay", in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic Press, New York, 73:147-166.

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRP) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review, see US 4275149 and US 4318980.

A label may be indirectly conjugated with an amino acid side chain, an acitivated amino acid side chain, a cysteine engineered antibody, and the like. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin or streptavidin, or vice versa. Biotin binds selectively to streptavidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the polypeptide variant, the polypeptide variant is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten polypeptide variant (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the polypeptide variant can be achieved (Hermanson, G. (1996) in Bioconjugate Techniques Academic Press, San Diego).

The antibody of the present invention may be employed in any known assay method, such as ELISA, competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Zola, (1987) Monoclonal Antibodies: A Manual of Techniques, pp.147-158, CRC Press, Inc.).

A detection label may be useful for localizing, visualizing, and quantitating a binding or recognition event. The labelled antibodies of the invention can detect cell-surface receptors. Another use for detectably labelled antibodies is a method of bead-based immunocapture comprising conjugating a bead with a fluorescent labelled antibody and detecting a fluorescence signal upon binding of a ligand. Similar binding detection methodologies utilize the surface plasmon resonance (SPR) effect to measure and detect antibody-antigen interactions.

Detection labels such as fluorescent dyes and chemiluminescent dyes (Briggs et al (1997) "Synthesis of Functionalised Fluorescent Dyes and Their Coupling to Amines and Amino Acids," J. Chem. Soc., Perkin-Trans. 1:1051-1058) provide a detectable signal and are generally applicable for labelling antibodies, preferably with the following properties: (i) the labelled antibody should produce a very high

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signal with low background so that small quantities of antibodies can be sensitively detected in both cell-free and cell-based assays; and (ii) the labelled antibody should be photostable so that the fluorescent signal may be observed, monitored and recorded without significant photo bleaching. For applications involving cell surface binding of labelled antibody to membranes or cell surfaces, especially live cells, the labels preferably (iii) have good water-solubility to achieve effective conjugate concentration and detection sensitivity and (iv) are non-toxic to living cells so as not to disrupt the normal metabolic processes of the cells or cause premature cell death.

Direct quantification of cellular fluorescence intensity and enumeration of fluorescently labelled events, e.g. cell surface binding of peptide-dye conjugates may be conducted on an system (FMAT® 8100 HTS System, Applied Biosystems, Foster City, Calif.) that automates mix-and-read, non-radioactive assays with live cells or beads (Miraglia, "Homogeneous cell- and bead-based assays for high throughput screening using fluorometric microvolume assay technology", (1999) J. of Biomolecular Screening 4:193-204). Uses of labelled antibodies also include cell surface receptor binding assays, inmmunocapture assays, fluorescence linked immunosorbent assays (FLISA), caspase-cleavage (Zheng, "Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo", (1998) Proc. Natl. Acad. Sci. USA 95:618-23; US 6372907), apoptosis (Vermes, "A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V" (1995) J. Immunol. Methods 184:39-51) and cytotoxicity assays. Fluorometric microvolume assay technology can be used to identify the up or down regulation by a molecule that is targeted to the cell surface (Swartzman, "A homogeneous and multiplexed immunoassay for high-throughput screening using fluorometric microvolume assay technology", (1999) Anal. Biochem. 271:143-51).

Labelled antibodies of the invention are useful as imaging biomarkers and probes by the various methods and techniques of biomedical and molecular imaging such as: (i) MRI (magnetic resonance imaging); (ii) MicroCT (computerized tomography); (iii) SPECT (single photon emission computed tomography); (iv) PET (positron emission tomography) Chen et al (2004) Bioconjugate Chem. 15:41-49; (v) bioluminescence; (vi) fluorescence; and (vii) ultrasound. Immunoscintigraphy is an imaging procedure in which antibodies labeled with radioactive substances are administered to an animal or human patient and a picture is taken of sites in the body where the antibody localizes (US 6528624). Imaging biomarkers may be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers may be of several types: Type 0 are natural history markers of a disease and correlate longitudinally with known clinical indices, e.g. MRI assessment of synovial inflammation in rheumatoid arthritis; Type I markers capture the effect of an intervention in accordance with a mechanism-of-action, even though the mechanism may not be associated with clinical outcome; Type II markers function as surrogate endpoints where the change in,

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or signal from, the biomarker predicts a clinical benefit to "validate" the targeted response, such as measured bone erosion in rheumatoid arthritis by CT. Imaging biomarkers thus can provide pharmacodynamic (PD) therapeutic information about: (i) expression of a target protein, (ii) binding of a therapeutic to the target protein, i.e. selectivity, and (iii) clearance and half-life pharmacokinetic data. Advantages of in vivo imaging biomarkers relative to lab-based biomarkers include: non-invasive treatment, quantifiable, whole body assessment, repetitive dosing and assessment, i.e. multiple time points, and potentially transferable effects from preclinical (small animal) to clinical (human) results. For some applications, bioimaging supplants or minimizes the number of animal experiments in preclinical studies.

Peptide labelling methods are well known. See Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, (1997) Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Glazer et al (1975) Chemical Modification of Proteins. Laboratory Techniques in Biochemistry and Molecular Biology (T. S. Work and E. Work, Eds.) American Elsevier Publishing Co., New York; Lundblad, R. L. and Noyes, C. M. (1984) Chemical Reagents for Protein Modification, Vols. I and II, CRC Press, New York; Pfleiderer, G. (1985) "Chemical Modification of Proteins", Modern Methods in Protein Chemistry, H. Tschesche, Ed., Walter DeGryter, Berlin and New York; and Wong (1991) Chemistry of Protein Conjugation and Cross-linking, CRC Press, Boca Raton, Fla.); De Leon-Rodriguez et al (2004) Chem.Eur. J. 10:1149-1155; Lewis et al (2001) Bioconjugate Chem. 12:320-324; Li et al (2002) Bioconjugate Chem. 13:110-115; Mier et al (2005) Bioconjugate Chem. 16:240-237.

Peptides and proteins labelled with two moieties, a fluorescent reporter and quencher in sufficient proximity undergo fluorescence resonance energy transfer (FRET). Reporter groups are typically fluorescent dyes that are excited by light at a certain wavelength and transfer energy to an acceptor, or quencher, group, with the appropriate Stokes shift for emission at maximal brightness. Fluorescent dyes include molecules with extended aromaticity, such as fluorescein and rhodamine, and their derivatives. The fluorescent reporter may be partially or significantly quenched by the quencher moiety in an intact peptide. Upon cleavage of the peptide by a peptidase or protease, a detectable increase in fluorescence may be measured (Knight, C. (1995) "Fluorimetric Assays of Proteolytic Enzymes", Methods in Enzymology, Academic Press, 248:18-34).

The labelled antibodies of the invention may also be used as an affinity purification agent. In this process, the labelled antibody is immobilized on a solid phase such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing the antigen to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the antigen to be purified, which is bound to the

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immobilized polypeptide variant. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the antigen from the polypeptide variant.

Labelling reagents typically bear reactive functionality which may react (i) directly with a cysteine thiol of a cysteine engineered antibody to form the labelled antibody, (ii) with a linker reagent to form a linker-label intermediate, or (iii) with a linker antibody to form the labelled antibody. Reactive functionality of labelling reagents include: maleimide, haloacetyl, iodoacetamide succinimidyl ester (e.g. NHS, N-hydroxysuccinimide), isothiocyanate, sulfonyl chloride, 2,6-dichlorotriazinyl, pentafluorophenyl ester, and phosphoramidite, although other functional groups can also be used.

An exemplary reactive functional group is N-hydroxysuccinimidyl ester (NHS) of a carboxyl group substituent of a detectable label, e.g. biotin or a fluorescent dye. The NHS ester of the label may be preformed, isolated, purified, and/or characterized, or it may be formed in situ and reacted with a nucleophilic group of an antibody. Typically, the carboxyl form of the label is activated by reacting with some combination of a carbodiimide reagent, e.g. dicyclohexylcarbodiimide, diisopropylcarbodiimide, or a uronium reagent, e.g. TSTU (O-(N-Succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate, HBTU (O-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), or HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate), an activator, such as 1-hydroxybenzotriazole (HOBt), and N-hydroxysuccinimide to give the NHS ester of the label. In some cases, the label and the antibody may be coupled by in situ activation of the label and reaction with the antibody to form the label-antibody conjugate in one step. Other activating and coupling reagents include TBTU (2-(1H-benzotriazo-1-yl)-1-1,3,3-tetramethyluronium hexafluorophosphate), TFFH (N,N',N",N'"-tetramethyluronium 2-fluoro-hexafluorophosphate), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline), DCC (dicyclohexylcarbodiimide); DIPCDI (diisopropylcarbodiimide), MSNT (1-(mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole, and aryl sulfonyl halides, e.g. triisopropylbenzenesulfonyl chloride.

Albumin binding peptide-Fab compounds of the invention:

In one aspect, the antibody of the invention is fused to an albumin binding protein. Plasma-protein binding can be an effective means of improving the pharmacokinetic properties of short lived molecules. Albumin is the most abundant protein in plasma. Serum albumin binding peptides (ABP) can alter the pharmacodynamics of fused active domain proteins, including alteration of tissue uptake, penetration, and diffusion. These pharmacodynamic parameters can be modulated by specific selection of the appropriate serum albumin binding peptide sequence (US 20040001827). A series of albumin binding peptides were identified by phage display screening (Dennis et al. (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" J Biol Chem. 277:35035-35043; WO 01/45746). Compounds of the invention include ABP sequences taught by: (i) Dennis et al (2002) J Biol Chem.

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277:35035-35043 at Tables III and IV, page 35038; (ii) US 20040001827 at [0076] SEQ ID NOS: 9-22; and (iii) WO 01/45746 at pages 12-13, all of which are incorporated herein by reference. Albumin Binding (ABP)-Fabs are engineered by fusing an albumin binding peptide to, for example, the C-terminus of Fab heavy chain in 1:1 stoichiometric ratio (1 ABP / 1 Fab). It was shown that association of these ABP-Fabs with albumin increased antibody half life by more than 25 fold in rabbits and mice. The above described reactive Cys residues can therefore be introduced in these ABP-Fabs and used for site-specific conjugation with cytotoxic drugs followed by in vivo animal studies.

Exemplary albumin binding peptide sequences include, but are not limited to, the amino acid sequences listed in SEQ ID NOS:80-84.

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	SEQ ID NO:80
QRLMEDICLPRWGCLWEDDF	SEQ ID NO:81
QRLIEDICLPRWGCLWEDDF	SEQ ID NO:82
RLIEDICLPRWGCLWEDD	SEQ ID NO:83
DICLPRWGCLW	SEQ ID NO:84

Antibody-Drug Conjugates

In another aspect, the invention provides immunoconjugates, or antibody-drug conjugates (ADC), comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). In another aspect, the invention further provides methods of using the immunoconjugates. In one aspect, an immunoconjugate comprises any of the above anti-STEAP-1 antibodies covalently attached to a cytotoxic agent or a detectable agent.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drg Del. Rev. 26:151-172; U.S. patent 4,975,278) allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., (1986) Lancet pp. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in Monoclonal Antibodies '84: Biological And Clinical Applications, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., (1986) Cancer Immunol. Immunother., 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., (1986) supra). Toxins used in antibody-toxin conjugates

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include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Mandler et al (2000) Jour. of the 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). The toxins may effect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition. Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al (2002) Blood 99(12):4336-42; Witzig et al (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; US Patent Nos. 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas) and cAC10 (specific to CD30 on hematological malignancies) (Doronina et al (2003) Nature Biotechnology 21(7):778-784) and are under therapeutic development.

Chemotherapeutic agents useful in the generation of immunoconjugates are described herein. Enzymatically active toxins and fragments thereof that 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,

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e.g., WO 93/21232 published October 28, 1993. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al (1987) Science, 238:1098. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (WO94/11026).

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

Maytansine and maytansinoids

In some embodiments, the immunoconjugate comprises an antibody (full length or fragments) of the invention conjugated to one or more maytansinoid molecules.

Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

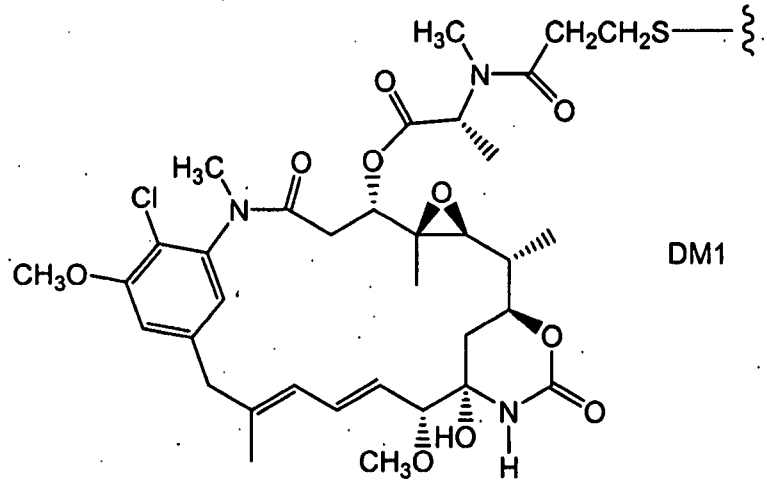
Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods.

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Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (US 4256746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (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

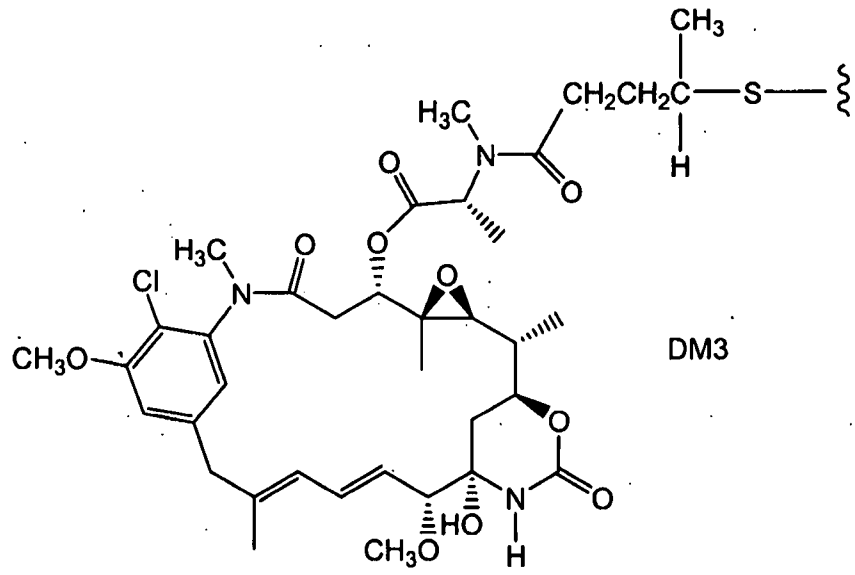
Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (US 4424219) (prepared by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (US 4450254) (prepared from Nocardia); C-15-hydroxy/acyloxy (US 4364866) (prepared by the conversion of maytansinol by Streptomyces); C-15-methoxy (US Pat. Nos. 4313946 and 4315929) (isolated from Trewia nudlflora); C-18-N-demethyl (US Pat. Nos. 4362663 and 4322348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (US 4371533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, having the structures:

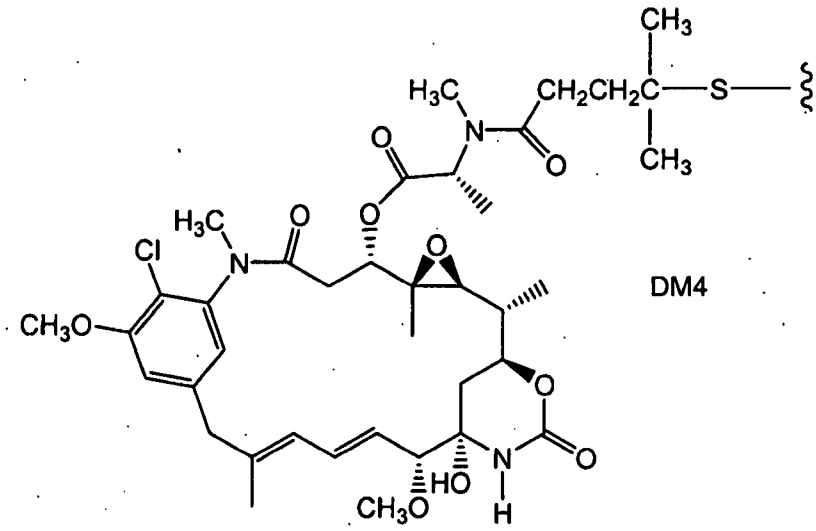


DM1

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DM3

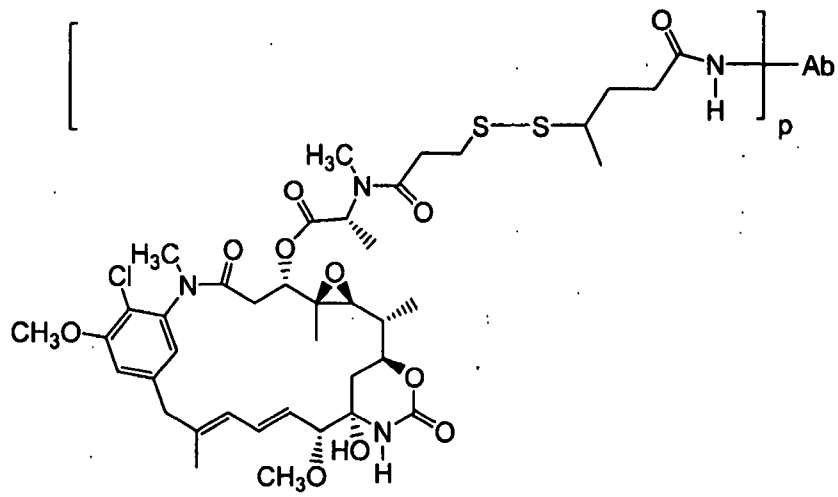


DM4

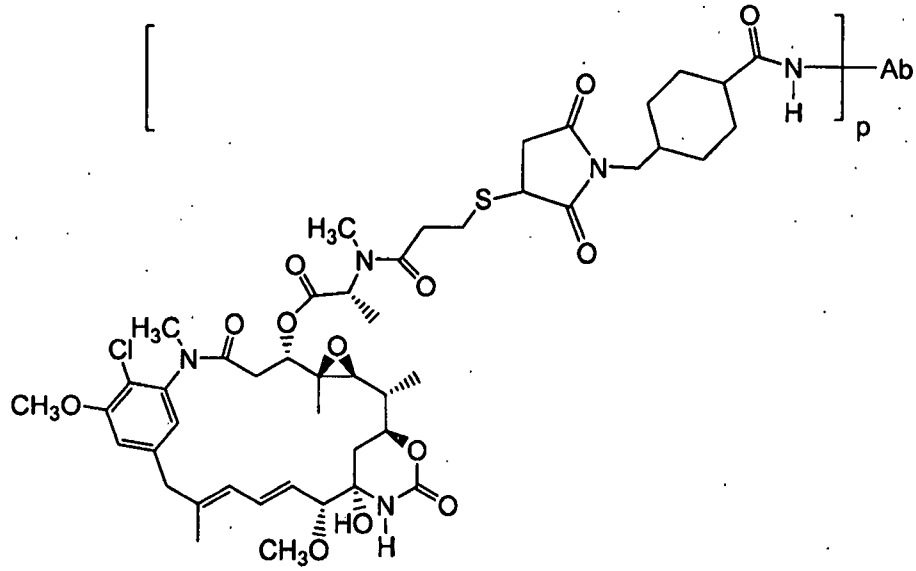
wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody drug conjugate. HERCEPTIN® (trastuzumab) linked by SMCC to DM1 has been reported (WO 2005/037992, which is expressly incorporated herein by reference in its entirety). An antibody drug conjugate of the present invention may be prepared according to the procedures disclosed therein.

Other exemplary maytansinoid antibody drug conjugates have the following structures and abbreviations, (wherein Ab is antibody and p is 1 to about 8):

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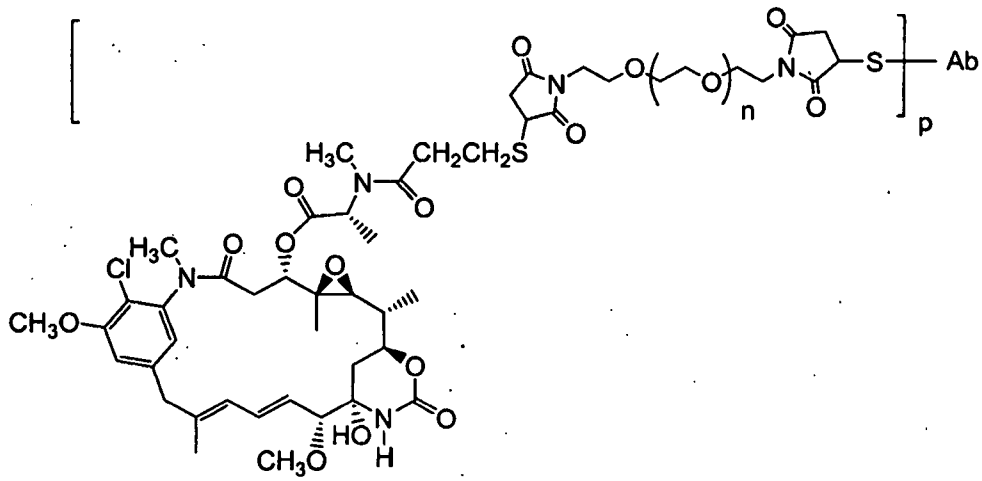


Ab -SPP-DM1



Ab-SMCC-DM1

Exemplary antibody drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1, 2, 3, or 4.

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Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020; 5,416,064; 6,441163 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 immunoconjugates 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. Chari et al., Cancer Research 52:127-131 (1992) describe immunoconjugates 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 3 x 10^5 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.

Anti-STEAP-1 antibody-maytansinoid conjugates are prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). An average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody, although even one molecule of toxin/antibody would be expected to enhance cytotoxicity over the use of naked antibody. Maytansinoids are well known in the art and can be synthesized by known techniques or isolated from natural sources. Suitable maytansinoids are disclosed, for example, in U.S. Patent No. 5,208,020 and in the other patents and nonpatent publications referred to hereinabove. Preferred maytansinoids are maytansinol and maytansinol analogues modified in the aromatic ring or at other positions of the maytansinol molecule, such as various maytansinol esters.

There are many linking groups known in the art for making antibody-maytansinoid conjugates, including, for example, those disclosed in U.S. Patent Nos. 5208020, 6441163, or EP Patent 0 425 235 B1, Chari et al., Cancer Research 52:127-131 (1992), and US 2005/0169933 A1, the disclosures of which are hereby expressly incorporated by reference. Antibody-maytansinoid conjugates comprising the linker component SMCC may be prepared as disclosed in U.S. Patent Application No. 11/141344, filed 31 May 2005, "Antibody Drug Conjugates and Methods". The linking groups include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups, or esterase labile groups, as disclosed in the above-identified patents. Additional linking groups are described and exemplified herein.

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Conjugates of the antibody and maytansinoid may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). Particularly preferred coupling agents include N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) (Carlsson et al., Biochem. J. 173:723-737 (1978)) and N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP) to provide for a disulfide linkage.

The linker may be attached to the maytansinoid molecule at various positions, depending on the type of the link. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. The reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In a preferred embodiment, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

In one embodiment, any of the antibodies of the invention (full length or fragment) is conjugated to one or more maytansinoid molecules. In one embodiment of the immunoconjugate, the cytotoxic agent D, is a maytansinoid DM1. In one embodiment of the immunoconjugate, the linker is selected from the group consisting of SPDP, SMCC, IT, SPDP, and SPP.

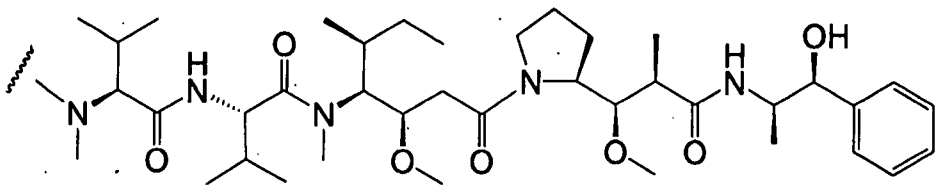
Auristatins and dolostatins

In some embodiments, the immunoconjugate comprises an antibody of the invention conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (US Patent Nos. 5635483; 5780588). 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 (US 5663149) 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).

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 March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

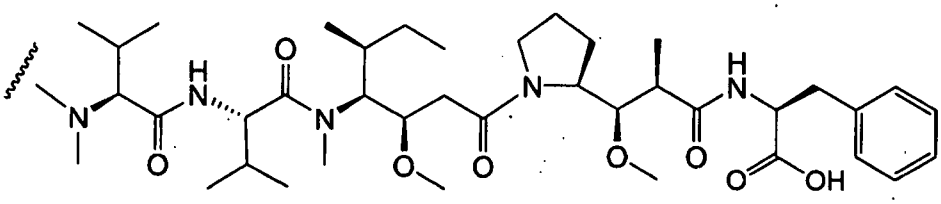
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An exemplary auristatin embodiment is MMAE (wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate).



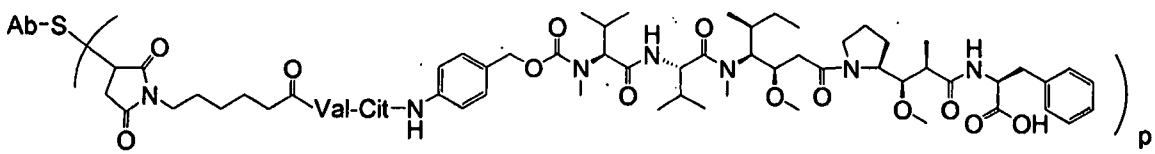
MMAE

Another exemplary auristatin embodiment is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate (US 2005/0238649):

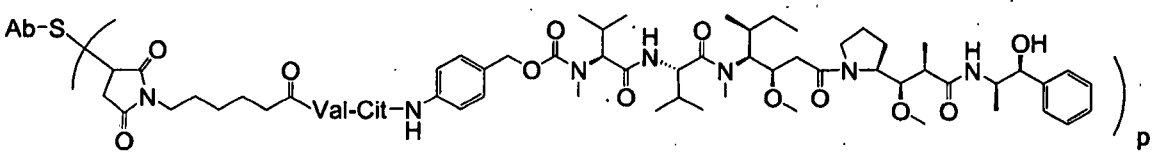


MMAF

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):

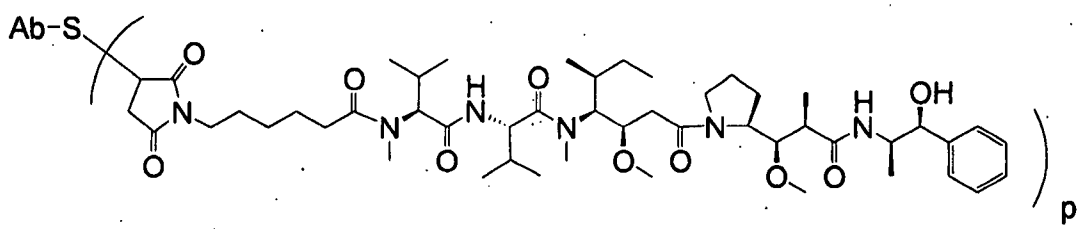


Ab-MC-vc-PAB-MMAF

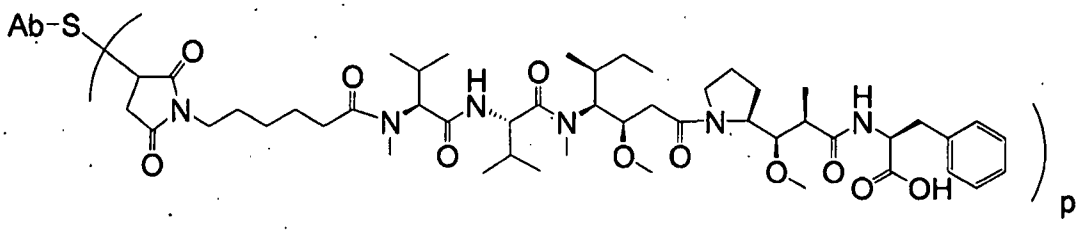


Ab-MC-vc-PAB-MMAE

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Ab-MC-MMAE



Ab-MC-MMAF

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. Schröder and K. Lübke, "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: US 5635483; US 5780588; 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

In other embodiments, the immunoconjugate comprises an antibody of the invention conjugated to one or more calicheamicin molecules. 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. patents 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, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman et al., Cancer Research 53:3336-3342 (1993), 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.

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Other cytotoxic agents

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. patents 5,053,394, 5,770,710, as well as esperamicins (U.S. patent 5,877,296).

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

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

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 At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the conjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc^{99m} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

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 tc^{99m} or I^{123}, .Re^{186}, Re^{188} and In^{111} 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.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For

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example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A). See pages 467-498, 2003-2004 Applications Handbook and Catalog.

Preparation of antibody drug conjugates:

In the antibody drug conjugates (ADC) of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), e.g. about 1 to about 20 drug moieties per antibody, through a linker (L). In one embodiment, the number of drug moieties (D) per antibody is from about 1 to about 5, alternatively, from about 2 to about 6, alternatively, from about 2 to about 5, alternatively from about 3 to about 4 drug moieties per antibody. Because the number of drug moieties per antibody is typically an average number over all conjugates in a population of an antibody drug conjugate, the number of drug moieties per antibody may not be a whole number. The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent, to form Ab-L, via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with the nucleophilic group of an antibody. Additional methods for preparing ADC are described herein.

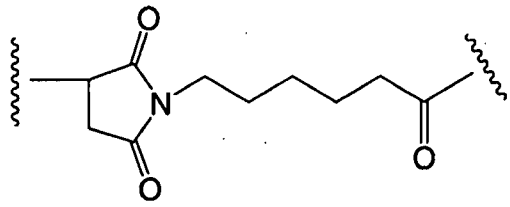
$$Ab–(L–D)_p \qquad \text{Formula I}$$

The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC'), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). In on embodiment, the linker is valine-citrullin-p-aminobenzyloxycaronyl ("vc-PAB"). Additional linker components are known in the art and some are described herein.

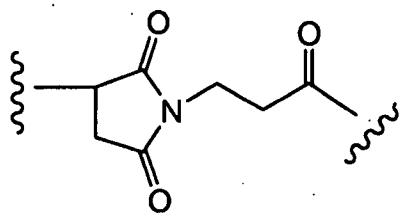
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In some embodiments, the linker may comprise amino acid residues. Exemplary amino acid linker components include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline. Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

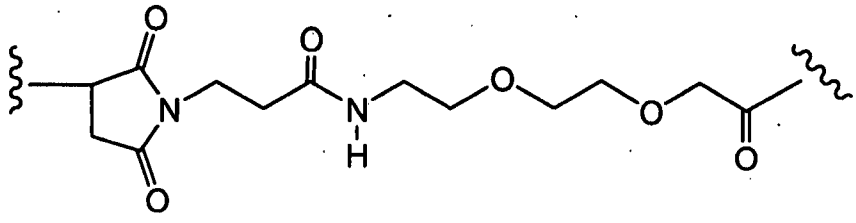
Exemplary linker component structures are shown below (wherein the wavy line indicates sites of covalent attachment to other components of the ADC):



MC



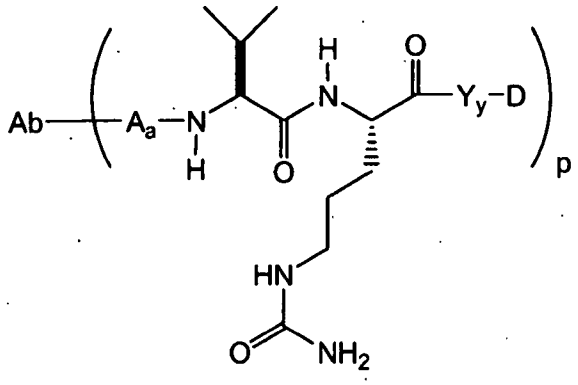
MP



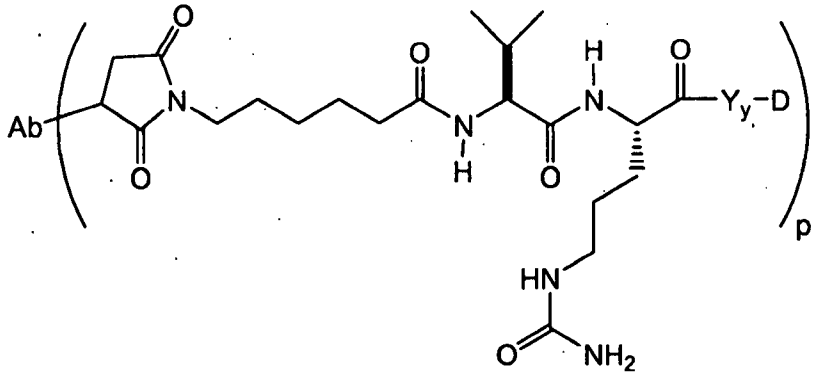
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Additional exemplary linker components and abbreviations include (wherein the antibody (Ab) and linker are depicted, and p is 1 to about 8):

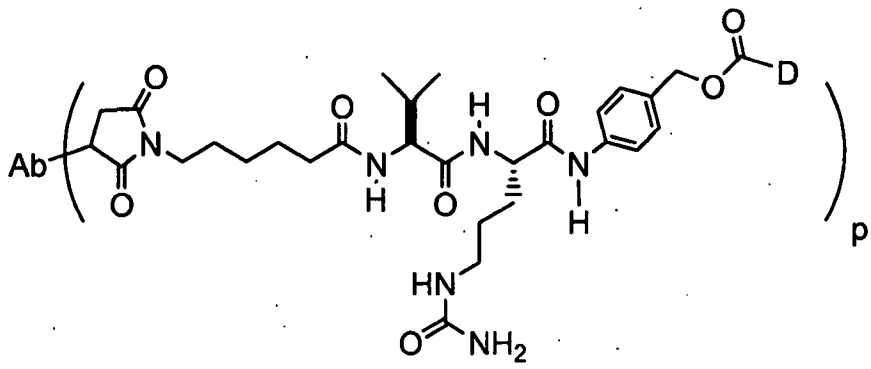
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Val-cit



MC-val-cit



MC-val-cit-PAB

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic

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groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by introducing one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody drug conjugates of the invention may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic subsituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either glactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the protein that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, proteins containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such aldehyde can be reacted with a drug moiety or linker nucleophile.

Likewise, nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

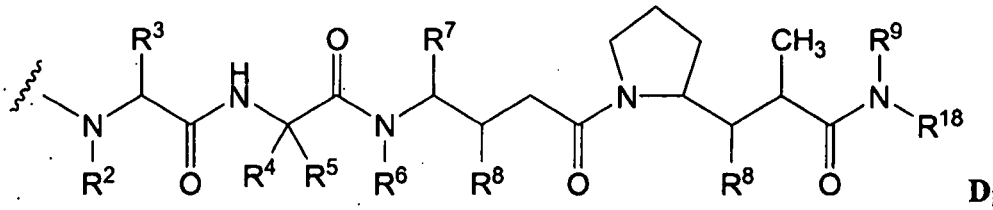
Methods for the conjugation of linker-drug moieties to cell-targeted proteins such as antibodies, immunoglobulins or fragments thereof are found, for example, in US5,208,020; US6,441,163; WO2005037992; WO2005081711; and WO2006/034488, all of which are hereby expressly incorporated by reference in their entirety.

Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

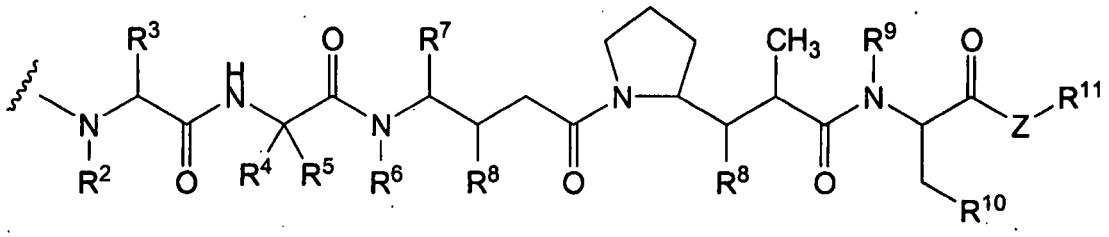
In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

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In one embodiment of the immunoconjugate, the cytotoxic agent, D, is an auristatin of formula D_E or D_F



D_E



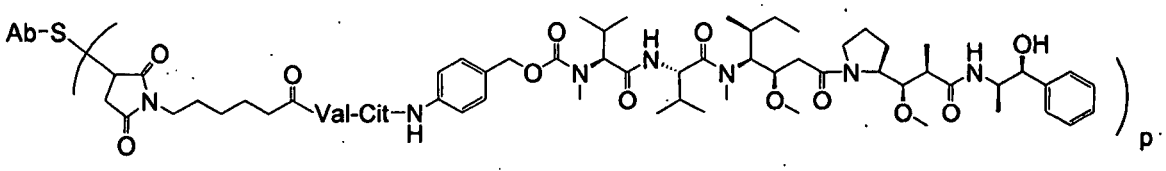
D_F

and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-CH_3$; and R^{18} is $-C(R^8)_2-C(R^8)_2-$aryl; and

(d) p ranges from about 1 to 8.

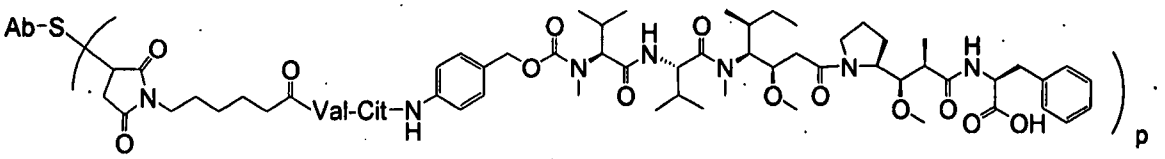
The following embodiments are further provided for any of the above immunoconjugates. In one embodiment, an immunoconjugate has in vitro or in vivo cell killing activity. In one embodiment, the linker is attached to the antibody through a thiol group on the antibody. In one embodiment, the linker is cleavable by a protease. In one embodiment, the linker comprises a val-cit dipeptide. In one embodiment, the linker comprises a p-aminobenzyl unit. In one embodiment, the p-aminobenzyl unit is disposed between the drug and a protease cleavage site in the linker. In one embodiment, the p-aminobenzyl unit is p-aminobenzyloxycarbonyl (PAB). In one embodiment, the linker comprises 6-maleimidocaproyl. In one embodiment, the 6-maleimidocaproyl is disposed between the antibody and a protease cleavage site in the linker. The above embodiments may occur singly or in any combination with one another.

In one embodiment, the drug is selected from MMAE and MMAF. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 2 to about 5. In one embodiment, the immunoconjugate has the formula

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wherein Ab is any of the above anti-STEAP-1 antibodies, S is a sulfur atom, and p ranges from about 1 to about 6, from about 2 to about 5, from about 2 to about 6, from about 2 to about 4, from about 2 to about 3, from about 3 to about 4, from about 3 to about 5, from about 3 to about 6, or from about 4 to about 6.

Labelled antibody imaging methods:

In another embodiment of the invention, cysteine engineered antibodies may be labelled through the cysteine thiol with radionuclides, fluorescent dyes, bioluminescence-triggering substrate moieties, chemiluminescence-triggering substrate moieties, enzymes, and other detection labels for imaging experiments with diagnostic, pharmacodynamic, and therapeutic applications. Generally, the labelled cysteine engineered antibody, i.e. "biomarker" or "probe", is administered by injection, perfusion, or oral ingestion to a living organism, e.g. human, rodent, or other small animal, a perfused organ, or tissue sample. The distribution of the probe is detected over a time course and represented by an image.

Articles of Manufacture:

In another embodiment of the invention, an article of manufacture, or "kit", containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds an antibody-drug conjugate (ADC) composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an ADC. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Pharmaceutical compositions:

In one aspect, a pharmaceutical composition is provided comprising any of the above immunoconjugates and a pharmaceutically acceptable carrier. In one aspect, a method of treating a prostate, lung, colon, bladder, or ovarian cell proliferative disorder, or Ewing's sarcoma is provided,

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wherein the method comprises administering to an individual the pharmaceutical composition. In one embodiment, the prostate, lung, colon, bladder, and ovarian cancer and Ewing's sarcoma cell proliferative disorder is a metastasis of a primary prostate, lung, colon, bladder, or ovarian cancer or Ewing's sarcoma. In one embodiment, the cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a cell.

In one aspect, a method of inhibiting cell proliferation is provided, wherein the method comprises exposing a cell to any of the above immunoconjugates under conditions permissive for binding of the immunoconjugate to STEAP-1. In one embodiment, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma is a tumor cell. In one embodiment, the tumor cell is a prostate, lung, colon, bladder, or ovarian tumor cell or Ewing's sarcoma celll of a mammal experiencing or suspected of experiencing prostate, lung, colon, bladder cell or Ewing's sarcoma proliferative disorder including, but not limited to, a metastasis of a primary prostate, lung, colon, bladder cell cancer tumor or Ewing's sarcoma tumor. In one embodiment, the prostate, lung, colon, bladder cell or Ewing's sarcoma is a xenograft. In one embodiment, the exposing takes place in vitro. In one embodiment, the exposing takes place in vivo.

In one aspect, a method of using the anti-STEAP-1 antibody of the invention is provided to assay serum soluble STEAP-1 in a mammal experiencing prostate lung or colon cell proliferative disorder (or metastasis of a primary incidence of such disorder), measuring clinical progression or regression of the diseases, or assess tumor burden or relapse.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the amino acid sequence of human STEAP-1 (SEQ ID NO:1) aligned with STEAP-1 from mouse and cynomolgus monkey (cyno) (SEQ ID NOs:2 and 3, respectively). Extracellular domains 1, 2, and 3 are labeled and marked by shaded boxes.

Figures 2A-2B: **Figure 2A** depicts the amino acid sequence of the light chain variable region of murine 120.545 anti-STEAP-1 antibody aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human subgroup III sequence. The CDRs are boxed (CDR-L1, CDR-L2, and CDR-L3). The sequences bracketing the CDRs are the framework sequences (FR-L1 to FR-L4). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs. **Figure 2B** depicts the amino acid sequence of the heavy chain variable region of murine anti-STEAP-1 antibody (120.545) aligned with the chimera antibody (120 chimera) and humanized antibody (120 graft) and aligned with the human kappa I sequence. Humanized variants 24, 37, 48, 67, and 37/48, 67, 71, and 78 were prepared by making the following amino acid changes: A24V, V37I, V48M, F67I, and L78F in the heavy chain of the 120 graft antibody. The CDRs are boxed. The FR-H1, FR-H2, FR-H3 and FR-H4 sequences bracket the CDRs (CDR-H1, CDR-H2, and

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CDR-H3). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed CDRs.

Figures 3A and 3B show exemplary acceptor human variable heavy (VH) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows, where the FR SEQ ID NOs are listed in the order FR-H1, FR-H2, FR-H3, FR-H4:

- human VH subgroup I consensus framework "A" minus Kabat CDRs (SEQ ID NOs:26, 27, 28, 29).

- human VH subgroup I consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:30, 31, 28, 29; SEQ ID NOs:30, 31, 32, 29; and SEQ ID NOs:30, 31, 33, 29).

- human VH subgroup II consensus framework "A" minus Kabat CDRs (SEQ ID NOs:34, 35, 36, 29).

- human VH subgroup II consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:37, 38, 36, 29; SEQ ID NOs:37, 38, 39, 29; and SEQ ID NOs:37, 38, 40, 29).

- human VH subgroup III consensus framework "A" minus Kabat CDRs (SEQ ID NOs:41, 42, 43, 29).

- human VH subgroup III consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 43, 29; SEQ ID NOs:44, 45, 46, 29; and SEQ ID NOs:44, 45, 46, 29).

- human VH acceptor 1 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 49, 29).

- human VH acceptor frameworks "B" and "C" minus extended hypervariable regions (SEQ ID NOs:44, 45, 49, 29; and SEQ ID NOs:44, 45, 50, 29).

- human VH acceptor 2 framework "A" minus Kabat CDRs (SEQ ID NOs:48, 42, 51, 29).

- human VH acceptor 2 framework "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:44, 45, 51, 29; SEQ ID NOs:44, 45, 52, 29; and SEQ ID NOs:44, 45, 53, 29).

Figures 4A and 4B show exemplary acceptor human variable light (VL) consensus framework sequences for use in practicing the instant invention with sequence identifiers as follows:

- human VL kappa subgroup I-1 consensus framework (κv1-1): SEQ ID NOs:54, 55, 56, 57

- human VL kappa subgroup I consensus framework (κv1): SEQ ID NOs:54, 58, 56, 57

- human VL kappa subgroup II consensus framework (κv2): SEQ ID NOs:58, 59, 60, 57

- human VL kappa subgroup III consensus framework (κv3): SEQ ID NOs:61, 62, 63, 57

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- human VL kappa subgroup IV consensus framework (κv4): SEQ ID NOs:64, 65, 66, 57.

Figure 5 depicts alignments of native sequence human IgG Fc region sequences, humIgG1 (non-A allotype, SEQ ID NO:85; and A allotype, where the amino acid sequence SREEM within SEQ ID NO:85 is changed to SRDEL), humIgG2 (SEQ ID NO:86), humIgG3 (SEQ ID NO:87) and humIgG4 (SEQ ID NO:88) with differences between the sequences marked with asterisks. Numbers above the sequences represent the EU numbering system. An exemplary kappa constant region is also shown.

Figures 6A-6D depicts a FACS analysis normalized for the level of display of each antibody or variant on phage. Figure 6A shows FACS shifts on STEAP-1 expressing cells (LB50) for four exemplary antibodies. Figure 6B shows FACS shifts on STEAP-1 non-expressing cells (S408) for several antibodies as indicated in the figure and in Example 1. Figures 6C and 6D are FACS shift alignments after normalization for phage display levels.

Figures 7A-7F graphically depict FACS analyses showing binding of anti-STEAP-1 murine, chimera and humanized version 24 antibodies to human STEAP-1 expressed on the cell surface. Figures 7A-7C indicate that anti-STEAP-1 murine 120, chimera 120 and humanized 120v.24 bind human and cynomolgus monkey STEAP-1, but not mouse STEAP-1. Figures 7D-7F are FACS plots showing binding of murine 120, 120 chimera, and humanized 120v.24 (clone 67) to human STEAP-1 expressed on the cell surface. Exogenous STEAP-1 was stably expressed in 293 cells (designated LB50 cells) and PC3 cells (designated PS5.4 cells) (Figures 7D and 7E), and endogenously expressed in LNCaP BR cells (Figure 7F).

Figures 8A and 8B. Figure 8A is a graph showing that administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. See Example 4. **Figure 8B** is a graph showing that single dose administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg), and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a LNCaP cell xenograft prostate tumor model. See Example 4.

Figure 9 is a graph showing that administration of anti-STEAP-1antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE) at 3 mg/kg, or anti-STEAP-1 120 chimera-MC-MMAF (abbreviated anti-STEAP mcMMAF) at 6 mg/kg, was shown to be efficacious in a prostate cancer xenograft model of castrated SCID-beige mice transplanted with LNCaP cells. See Example 4.

Figure 10 is a graph showing that administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (abbreviated anti-STEAP vcMMAE (at 3 mg/kg) was shown to be efficacious in a prostate

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cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. See Example 4.

Figure 11 is a graph showing that administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, humanized anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. See Example 4.

Figure 12 is a diagram depicting STEAP-1 embedded in a cell membrane. Anti-STEAP-1 antibody 120 binding is conformation dependent and does not recognize a linear epitope of STEAP-1.

Figures 13A-13D show STEAP-1 expressed on the surface of cells as detected by immunohistochemistry. **Figure 13A** shows an immunohistochemical stain of 293 cells expressing exogenous STEAP-1 on the cell surface. **Figure 13B** shows an immunohistochemical stain of PC3 cells expressing exogenous STEAP-1 on the cell surface. **Figure 13C** shows an immunohistochemical stain of LNCaP cells expressing endogenous STEAP-1 on the cell surface. **Figure 13D** shows an immunohistochemica stain of LuCAP 77 cells expressing endogenous STEAP-1 on the cell surface.

Figures 14A-14E are graphs showing the relative effectiveness of anti-STEAP-1 antibody 120v.24-MCMMAF and anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE to kill STEAP-1 expressing cells in vitro. PS5.4 cells (**Figure 14A**) are PC3 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. LB50 cells (**Figure 14B**) are 293 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. LNCaP cells (**Figure 14C**) express STEAP-1 endogenously. "PC3 vec" (**Figure 14D**) and "293 vec" (**Figure 14E**) refer to 293 cells and PC3 cells, respectively, transformed with a vector control.

Figure 15 shows depictions of cysteine engineered anti-STEAP-1 antibody drug conjugates (ADC) where a drug moiety is attached to an engineered cysteine group in: the light chain (LC-ADC); the heavy chain (HC-ADC); and the Fc region (Fc-ADC).

Figure 16 shows the steps of: (i) reducing cysteine disulfide adducts and interchain and intrachain disulfides in a cysteine engineered anti-STEAP-1 antibody (ThioMab) with reducing agent TCEP (tris(2-carboxyethyl)phosphine hydrochloride); (ii) partially oxidizing, i.e. reoxidation to reform interchain and intrachain disulfides, with dhAA (dehydroascorbic acid); and (iii) conjugation of the reoxidized antibody with a drug-linker intermediate to form a cysteine engineered anti-STEAP-1 antibody drug conjugate (ADC).

Figures 17A-C show the sites of amino acid substitutions made to generate cysteine-engineered anti-STEAP-1 antibodies (thio-mAbs). **Figure 17A** shows the thio-LC variant V205C with corresponding

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sequential numbering and standardized numbering according to the Kabat system. **Figure 17B** shows the thio-HC variant A121C with corresponding sequential numbering and standardized numbering according to the EU system. **Figure 17C** shows the thio-Fc variant A121CS400C with corresponding sequential numbering and standardized numbering according to the EU system.

Figures 18A-F depict FACS analyses showing that the anti-STEAP-1 thio antibody drug conjugates (TDCs) retain the ability to bind to STEAP-1 expressed on the cell surface. **Figures 18A-18C** are FACS plots showing binding of the anti-STEAP-1 TDCs thio-human120-vc-PAB-MMAE (LCV205C) (abbreviated as huSteap1 TDC (L211C) vcE and thio-human120-vc-PAB-MMAE (HCA118C) (abbreviated as huSteap1 TDC (HCA125C) vcE) to human STEAP-1 expressed on the cell surface. Exogenous STEAP-1 was stably expressed in 293 cells (designated LB50 cells) and PC3 cells (designated PS5.4 cells) (**Figures 18A and 18B**), and endogenously expressed in LNCaP BR cells (**Figure 18C**). **Figures 18D, 18E and 18F** are alignments of the FACS shifts shown in **Figure 7A, 7B and 7C**, respectively.

Figures 19A-C show the relative effectiveness of the anti-STEAP-1 thio antibody drug conjugates (TDCs) thio-human120-vc-PAB-MMAE (LCV211C) (abbreviated as huSteap1 TDC (L211C) vcE and thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 TDC (HCA125C) vcE)to kill STEAP-1 expressing cells in vitro. LB50 cells (**Figure 19A**) are 293 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. PS5.4 cells (**Figure 19B**) are PC3 cells transformed with a vector encoding STEAP-1 such that STEAP-1 is expressed on the cell surface. LNCaP cells (**Figure 19C**) express STEAP-1 endogenously.

Figure 20 is a graph showing that administration of anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as hu Steap1 HC TDC vcE) at 3 mg/kg was shown to be efficacious relative to controls in a prostate cancer xenograft model of male SCID-beige mice (androgen-dependent) transplanted with LNCaP cells. See Example 8.

Figure 21 is a graph showing that administration of anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as hu Steap1 HC TDC vcE) at 3 mg/kg, or thio-human120-MC-MMAF (HCA125C) (abbreviated as hu Steap1 HC TDC mcF) at 1, 3 or 6 mg/kg, was shown to be efficacious relative to controls in a prostate cancer xenograft model of male SCID-beige mice (androgen-dependent) transplanted with LNCaP cells. See Example 8.

Figure 22 is a graph showing that administration of anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as hu Steap1 HC TDC vcE) at 3 mg/kg, or thio-human120-MC-MMAF (HCA125C) (abbreviated as hu Steap1 HC TDC mcF) at 3 or 6 mg/kg, was shown to be efficacious

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relative to controls in a prostate cancer xenograft model of castrated SCID-beige mice transplanted with LuCaP 35V prostate tumor. See Example 8.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Isolated antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are further provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as a tumor or cancer. In certain embodiments, STEAP-1 is expressed in tumor or cancer of prostate, lung, or colon tissue. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1, e.g., STEAP-1 expressed on the cell surface. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the detection of STEAP-1 expression on the surface of normal and/or tumor or cancer cells of protate, lung or colon tissue.

Polynucleotides encoding anti-STEAP-1 antibodies are provided. Vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided, and host cells comprising such vectors are provided. Compositions, including pharmaceutical formulations, comprising any one or more of the polynucleotides, anti-STEAP-1 antibodies, or immunoconjugates of the invention are also provided.

Methods of treating a cell proliferative disorder, including but not limited to tumor or cancer, with an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, treatment of tumor or cancer in prostate, lung or colon of a mammal. Methods of detecting STEAP-1 expression on a tissue cell using an anti-STEAP-1 antibody, antibody drug conjugate or immunoconjugate are provided. Such methods include, but are not limited to, detection of STEAP-1 expression on, as a non-limiting example, a normal cell, tumor cell, or cancer cell of prostate, lung, or colon cell.

General Techniques

The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds., (2003)); the series Methods in Enzymology (Academic Press, Inc.): Pcr 2: A Practical Approach (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture (R. I. Freshney, ed. (1987)); Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana

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Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney), ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V. T. DeVita et al., eds., J.B. Lippincott Company, 1993).

DEFINITIONS AND ABBREVIATIONS

Definitions

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

An "isolated" nucleic acid molecule is a nucleic acid molecule that is separated from at least one other nucleic acid molecule with which it is ordinarily associated, for example, in its natural environment. An isolated nucleic acid molecule further includes a nucleic acid molecule contained in cells that ordinarily express the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Purified" means that a molecule is present in a sample at a concentration of at least 95% by weight, or at least 98% by weight of the sample in which it is contained.

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The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (e.g., Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors," or simply, "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present,

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modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

"Oligonucleotide," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if

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necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "STEAP-1," as used herein, refers to any native STEAP-1 from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed STEAP-1 as well as any form of STEAP-1 that results from processing in the cell. The term also encompasses naturally occurring variants of STEAP-1, e.g., splice variants, allelic variants, and isoforms. The amino acid sequence of human STEAP-1 is depicted in Figure 1 (SEQ ID NO:1). In one embodiment, STEAP-1 is expressed on the cell surface, such as on the surface of a normal prostate, lung or colon cell, and has increased expression in prostate, lung or colon cancer cells or metastases of such cancer cells. Figure 1

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also depicts the amino acid sequence of STEAP-1 from mouse and cynomolgus monkey (SEQ ID NOs:2 and 3, respectively).

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having similar structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which generally lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (e.g., full length or intact monoclonal antibodies), polyclonal antibodies, monovalent antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be chimeric, human, humanized and/or affinity matured.

The term "anti-STEAP-1 antibody" or "an antibody that binds to STEAP-1" refers to an antibody that is capable of binding STEAP-1 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting STEAP-1. Preferably, the extent of binding of an anti-STEAP-1 antibody to an unrelated, non-STEAP-1 protein is less than about 10% of the binding of the antibody to STEAP-1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to STEAP-1 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an anti-STEAP-1 antibody binds to an epitope of STEAP-1 that is conserved among STEAP-1 from different species.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by

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the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

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

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain the Fc region.

"Antibody fragments" comprise only a portion of an intact antibody, wherein the portion retains at least one, and as many as most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an in vivo half life substantially similar to an intact antibody. For example, such an antibody fragment may comprise on antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

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

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"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO93/1161; Hudson et al. (2003) Nat. Med. 9:129-134; and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al. (2003) Nat. Med. 9:129-134.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor

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amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler et al., Nature, 256: 495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage display technologies (see, e.g., Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132(2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893; WO96/34096; WO96/33735; WO91/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks et al., Bio.Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996) and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

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The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six hypervariable regions, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. Xu et al. (2000) Immunity 13:37-45; Johnson and Wu (2003) in Methods in Molecular Biology 248:1-25

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(Lo, ed., Human Press, Totowa, NJ). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. Hamers-Casterman et al. (1993) Nature 363:446-448; Sheriff et al. (1996) Nature Struct. Biol. 3:733-736.

A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below.

Loop	Kabat	AbM	Chothia	Contact	
---	---	---	---	---	
L1	L24-L34	L24-L34	L26-L32	L30-L36	
L2	L50-L56	L50-L56	L50-L52	L46-L55	
L3	L89-L97	L89-L97	L91-L96	L89-L96	
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B	(Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35	(Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58	
H3	H95-H102	H95-H102	H96-H101	H93-H101	

Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al., supra, for each of these definitions. The HVR-H1, HVR-H2, and HVR-H3 hypervariable regions of the humanized anti-STEAP-1 120v.24 antibodies of the invention are H26-H35A, H49-H6, and H95-H102 using Kabat numbering. The HVR-L1, HVR-L2, and HVR-L3 hypervariable regions of the humanized anti-STEP-1 120v.24 antibodies of the invention are L24-34, L50-56, and L89-97 using Kabat numbering. As used herein, the terms "HVR" and "CDR" are used interchangeably.

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"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

An "affinity matured" antibody is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In one embodiment, an affinity matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. Bio/Technology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of HVR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci. USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

A "blocking" antibody or an "antagonist" antibody is one which inhibits or reduces biological activity of the antigen it binds. Certain blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

An "agonist antibody," as used herein, is an antibody which mimics at least one of the functional activities of a polypeptide of interest.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one which binds an IgG

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antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, e.g., Ghetie 1997, Hinton 2004). Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates administered with the Fc variant polypeptides.

WO00/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. The content of that patent publication is specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a native source, e.g., from blood.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 or Presta U.S. Patent No. 6,737,056 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells

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(PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

"Complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed.

Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US Patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

The term "Fc region-comprising polypeptide" refers to a polypeptide, such as an antibody or immunoadhesin, which comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the polypeptide or by recombinant engineering the nucleic acid encoding the polypeptide. Accordingly, a composition comprising a polypeptide having an Fc region according to this invention can comprise polypeptides with K447, with all K447 removed, or a mixture of polypeptides with and without the K447 residue.

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferably those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may be 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

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In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "VH subgroup III consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat et al., supra. In one embodiment, the VH subgroup III consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: EVQLVESGGGLVQPGGSLRLSCAAS (FR-H1, SEQ ID NO:21)-HVR-H1-WVRQAPGKGLEWV (FR-H2, SEQ ID NO:22)-HVR-H2-RFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR (FR-H3, SEQ ID NO:23)-HVR-H3-WGQGTLVTVSS (FR-H4, SEQ ID NO:24).

A "VL subgroup I consensus framework" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat et al., supra. In one embodiment, the VH subgroup I consensus framework amino acid sequence comprises at least a portion or all of each of the following sequences: DIQMTQSPSSLSASVGDRVTITC (FR-L1, SEQ ID NO:17)-HVR-L1-WYQQKPGKAPKLLIY (FR-L2, SEQ ID NO:18)-HVR-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, SEQ ID NO:19)-HVR-L3-FGQGTKVEIKR (FR-L4, SEQ ID NO:20). "Secretion signal sequence" or "signal sequence" refers to a nucleic acid sequence encoding a short signal peptide that can be used to direct a newly synthesized protein of interest through a cellular membrane, usually the inner membrane or both inner and outer membranes of prokaryotes. As such, the protein of interest such as the immunoglobulin light or heavy chain polypeptide is secreted into the periplasm of the prokaryotic host cells or into the culture medium. The signal peptide encoded by the secretion signal sequence may be endogenous to the host cells, or they may be exogenous, including signal peptides native to the polypeptide to be expressed. Secretion signal sequences are typically present at the amino terminus of a polypeptide to be expressed, and are typically removed enzymatically between biosynthesis and secretion of the polypeptide from the cytoplasm. Thus, the signal peptide is usually not present in a mature protein product.

A "free cysteine amino acid" refers to a cysteine amino acid residue which has been engineered into a parent antibody, has a thiol functional group (-SH), and is not paired as, or otherwise part of, an intramolecular or intermolecular disulfide bridge.

The term "thiol reactivity value" is a quantitative characterization of the reactivity of free cysteine amino acids. The thiol reactivity value is the percentage of a free cysteine amino acid in a cysteine engineered antibody which reacts with a thiol-reactive reagent, and converted to a maximum value of 1. For example, a free cysteine amino acid on a cysteine engineered antibody which reacts in 100% yield with a thiol-reactive reagent, such as a biotin-maleimide reagent, to form a biotin-labelled antibody has a thiol reactivity value of 1.0. Another cysteine amino acid engineered into the same or different parent

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antibody which reacts in 80% yield with a thiol-reactive reagent has a thiol reactivity value of 0.8. Another cysteine amino acid engineered into the same or different parent antibody which fails totally to react with a thiol-reactive reagent has a thiol reactivity value of 0. Determination of the thiol reactivity value of a particular cysteine may be conducted by ELISA assay, mass spectroscopy, liquid chromatography, autoradiography, or other quantitative analytical tests. Thiol-reactive reagents which allow capture of the cysteine engineered antibody and comparison and quantitation of the cysteine reactivity include biotin-PEO-maleimide ((+)-biotinyl-3-maleimidopropionamidyl-3,6-dioxaoctainediamine, Oda et al (2001) Nature Biotechnology 19:379-382, Pierce Biotechnology, Inc.) Biotin-BMCC, PEO-Iodoacetyl Biotin, Iodoacetyl-LC-Biotin, and Biotin-HPDP (Pierce Biotechnology, Inc.), and Nα-(3- maleimidylpropionyl)biocytin (MPB, Molecular Probes, Eugene, OR). Other commercial sources for biotinylation, bifunctional and multifunctional linker reagents include Molecular Probes, Eugene, OR, and Sigma, St. Louis, MO

A "parent antibody" is an antibody comprising an amino acid sequence from which one or more amino acid residues are replaced by one or more cysteine residues. The parent antibody may comprise a native or wild type sequence. The parent antibody may have pre-existing amino acid sequence modifications (such as additions, deletions and/or substitutions) relative to other native, wild type, or modified forms of an antibody. A parent antibody may be directed against a target antigen of interest, e.g. a biologically important polypeptide. Antibodies directed against nonpolypeptide antigens (such as tumor-associated glycolipid antigens; see US 5091178) are also contemplated.

"Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative embodiments are described in the following.

In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (125I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (Chen, et

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al., (1999) J. Mol. Biol. 293:865-881). To establish conditions for the assay, microtiter plates (Dynex) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., (1997) Cancer Res. 57:4593-4599). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% Tween-20 in PBS. When the plates have dried, 150 μl/well of scintillant (MicroScint-20; Packard) is added, and the plates are counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, the Kd or Kd value is measured by using surface plasmon resonance assays using a BIAcoreTM-2000 or a BIAcoreTM-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% Tween 20 (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881. If the on-rate exceeds 106 M-1 s-1 by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25oC of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-Aminco spectrophotometer (ThermoSpectronic) with a stirred cuvette.

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An "on-rate," "rate of association," "association rate," or "k_{on}" according to this invention can also be determined as described above using a BIAcore™-2000 or a BIAcore™-3000 system (BIAcore®, Inc., Piscataway, NJ).

A "disorder" is any condition or disease that would benefit from treatment with an substance/molecule or method of the invention. This includes chronic and acute disorders including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancerous conditions such as cancers or metastases of prostate, lung, and colon.

The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

"Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, multiple myeloma and B-cell lymphoma, brain , as well as head and neck cancer, and associated metastases.

A "STEAP-1-expressing cell" is a cell which expresses endogenous or transfected STEAP-1 on the cell surface. A "STEAP-1-expressing cancer" is a cancer comprising cells that have STEAP-1 protein present on the cell surface. A "STEAP-1-expressing cancer" produces sufficient levels of STEAP-1 on the surface of cells thereof, such that an anti-STEAP-1 antibody can bind thereto and have a therapeutic effect with respect to the cancer. A cancer which "overexpresses" STEAP-1 is one which has significantly higher levels of STEAP-1 at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or

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translation. STEAP-1 overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the STEAP-1 protein present on the surface of a cell (e.g. via an immunohistochemistry assay; FACS analysis). Alternatively, or additionally, one may measure levels of STEAP-1-encoding nucleic acid or mRNA in the cell, e.g. via fluorescent in situ hybridization; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study STEAP-1 overexpression by measuring shed antigen in a biological fluid such as serum, e.g, using antibody-based assays (see also, e.g., U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias et al. J. Immunol. Methods 132: 73-80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody. A STEAP-1-expressing cancer includes prostate, lung, and colon cancer.

As used herein, "treatment" (and variations such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or disorder or to slow the progression of a disease or disorder.

The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician. For cancer therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). For prostate cancer, the progress of therapy can be assessed by routine methods, usually by measuring serum PSA (prostate specific antigen) levels; the higher the level of PSA in the blood, the more extensive the cancer. Commercial assays for detecting PSA are available, e.g, Hybitech Tandem-E and Tandem-R PSA assay kits, the Yang ProsCheck polyclonal assay (Yang Labs, Bellevue, WA), Abbott Imx (Abbott Labs, Abbott Park, IL), etc. Metastasis can be determined by staging tests and by bone scan and tests for calcium level and other enzymes to determine spread to the bone. CT scans can also be done to look for spread to the pelvis and lymph nodes in the area. Chest X-rays and measurement of liver enzyme levels by known methods are used to look for metastasis to the lungs and liver,

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respectively. Other routine methods for monitoring the disease include transrectal ultrasonography (TRUS) and transrectal needle biopsy (TRNB).

An "individual" is a vertebrate. In certain embodiments, the vertebrate is a mammal. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as cats, dogs, and horses), primates, mice and rats. In certain embodiments, a mammal is a human.

An "effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of a substance/molecule of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating". To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine;

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monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Label" as used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

The term "epitope tagged" used herein refers to a chimeric polypeptide comprising an anti-PSCA antibody polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the Ig polypeptide to which it is fused. The tag polypeptide is also preferably fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated nucleic acid" is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

"Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (*e.g.*, the ColE1 origin of replication) and a selectable marker (*e.g.*, ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the invention, in bacterial or eukaryotic cells. Suitable vectors are disclosed below.

The cell that produces an anti-STEAP-1 antibody of the invention will include the parent hybridoma cell e.g., the hybridomas that are deposited with the ATCC, as well as bacterial and eukaryotic

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host cells into which nucleic acid encoding the antibodies have been introduced. Suitable host cells are disclosed below.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially a PSCA expressing cancer cell, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of PSCA expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu), chemotherapeutic agents (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, toxins, growth inhibitory agents, drug moieties, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

A "toxin" is any substance capable of having a detrimental effect on the growth or proliferation of a cell.

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

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trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaI1 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C");

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thiotepa; taxoids, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® docetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), EVISTA® raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; anti-progesterones; estrogen receptor down-regulators (ERDs); agents that function to suppress or shut down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as LUPRON® and ELIGARD® leuprolide acetate, goserelin acetate, buserelin acetate and tripterelin; other anti-androgens such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole. In addition, such definition of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), DIDROCAL® etidronate, NE-58095, ZOMETA® zoledronic acid/zoledronate, FOSAMAX® alendronate, AREDIA® pamidronate, SKELID® tiludronate, or ACTONEL® risedronate; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; lapatinib ditosylate (an ErbB-2 and

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EGFR dual tyrosine kinase small-molecule inhibitor also known as GW572016); and pharmaceutically, acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell (such as a cell expressing STEAP-1) either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of cells (such as a cell expressing STEAP-1) in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in The Molecular Basis of Cancer, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "intracellular metabolite" refers to a compound resulting from a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC). The metabolic process or reaction may be an enzymatic process, such as proteolytic cleavage of a peptide linker of the ADC, or hydrolysis of a functional group such as a hydrazone, ester, or amide. Intracellular metabolites include, but are not limited to, antibodies and free drug which have undergone intracellular cleavage after entry, diffusion, uptake or transport into a cell.

The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction inside a cell on an antibody-drug conjugate (ADC) whereby the covalent attachment, i.e. linker, between the drug moiety (D) and the antibody (Ab) is broken, resulting in the free drug dissociated from the antibody inside the cell. The cleaved moieties of the ADC are thus intracellular metabolites.

The term "bioavailability" refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

The term "cytotoxic activity" refers to a cell-killing, cytostatic or growth inhibitory effect of an antibody-drug conjugate or an intracellular metabolite of an antibody-drug conjugate. Cytotoxic activity

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may be expressed as the IC_{50} value, which is the concentration (molar or mass) per unit volume at which half the cells survive.

"Alkyl" is C1-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH3), ethyl (Et, -CH2CH3), 1-propyl (n-Pr, n-propyl, -CH2CH2CH3), 2-propyl (i-Pr, i-propyl, -CH(CH3)2), 1-butyl (n-Bu, n-butyl, -CH2CH2CH2CH3), 2-methyl-1-propyl (i-Bu, i-butyl, -CH2CH(CH3)2), 2-butyl (s-Bu, s-butyl, -CH(CH3)CH2CH3), 2-methyl-2-propyl (t-Bu, t-butyl, -C(CH3)3), 1-pentyl (n-pentyl, -CH2CH2CH2CH2CH3), 2-pentyl (-CH(CH3)CH2CH2CH3), 3-pentyl (-CH(CH2CH3)2), 2-methyl-2-butyl (-C(CH3)2CH2CH3), 3-methyl-2-butyl (-CH(CH3)CH(CH3)2), 3-methyl-1-butyl (-CH2CH2CH(CH3)2), 2-methyl-1-butyl (-CH2CH(CH3)CH2CH3), 1-hexyl (-CH2CH2CH2CH2CH2CH3), 2-hexyl (-CH(CH3)CH2CH2CH2CH3), 3-hexyl (-CH(CH2CH3)(CH2CH2CH3)), 2-methyl-2-pentyl (-C(CH3)2CH2CH2CH3), 3-methyl-2-pentyl (-CH(CH3)CH(CH3)CH2CH3), 4-methyl-2-pentyl (-CH(CH3)CH2CH(CH3)2), 3-methyl-3-pentyl (-C(CH3)(CH2CH3)2), 2-methyl-3-pentyl (-CH(CH2CH3)CH(CH3)2), 2,3-dimethyl-2-butyl (-C(CH3)2CH(CH3)2), 3,3-dimethyl-2-butyl (-CH(CH3)C(CH3)3.

The term "C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative "C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl,-acetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, -3-methyl-1 butynyl. methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isoheptyl, n-octyl, and isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Alkenyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH_2), allyl (-CH_2CH=CH_2), cyclopentenyl (-C_5H_7), and 5-hexenyl (-CH_2 $CH_2CH_2CH_2$CH=CH_2)

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"Alkynyl" is C2-C18 hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic (-C≡CH) and propargyl (-CH_2C≡CH),

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH_2-) 1,2-ethyl (-CH_2CH_2-), 1,3-propyl (-$CH_2CH_2CH_2$-), 1,4-butyl (-$CH_2CH_2CH_2CH_2$-), and the like.

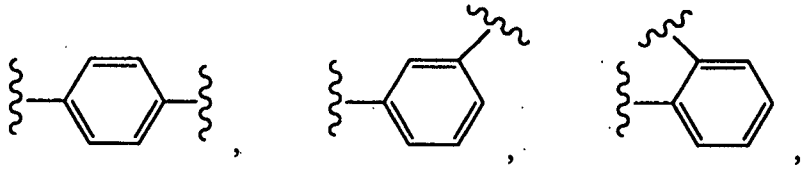
A "C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula -$(CH_2)_{1-10}$-. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or a heterocyclic aromatic group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2, -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -N(R')$_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:



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in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2R'$, -S(O)R', -OH, -halogen, -N_3, -NH_2, -NH(R'), -$N(R')_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

"Substituted alkyl," "substituted aryl," and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, $-O^-$, -OR, -SR, $-S^-$, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, $=N_2$, -N_3, NC(=O)R, -C(=O)R, -$C(=O)NR_2$, -SO_3^-, -SO_3H, -$S(=O)_2R$, -$OS(=O)_2OR$, -$S(=O)_2NR$, -S(=O)R, -$OP(=O)(OR)_2$, -$P(=O)(OR)_2$, -PO^-_3, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2^-, -C(=S)OR, -C(=O)SR, -C(=S)SR, -$C(=O)NR_2$, -$C(=S)NR_2$, -$C(=NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, C_2-C_{18} alkyl, C_6-C_{20} aryl, C_3-C_{14} heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

"Heteroaryl" and "heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

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Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

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A "C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; wherein each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

A "C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -aryl, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -$S(O)_2$R', -S(O)R', -OH, -halogen, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ and -CN; where each R' is independently selected from H, -C_1-C_8 alkyl and aryl.

A "C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

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"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, linkers include a divalent radical such as an alkyldiyl, an aryldiyl, a heteroaryldiyl, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

"Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to, a halide

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(e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

Abbreviations

LINKER COMPONENTS:

MC = 6-maleimidocaproyl

Val-Cit or "vc" = valine-citrulline (an exemplary dipeptide in a protease cleavable linker)

Citrulline = 2-amino-5-ureido pentanoic acid

PAB = p-aminobenzyloxycarbonyl (an example of a "self immolative" linker component)

Me-Val-Cit = N-methyl-valine-citrulline (wherein the linker peptide bond has been modified to prevent its cleavage by cathepsin B)

MC(PEG)6-OH = maleimidocaproyl- polyethylene glycol (can be attached to antibody cysteines).

SPP = N-succinimidyl-4-(2-pyridylthio)pentanoate

SPDP = N-succinimidyl-3-(2-pyridyldithio) propionate

SMCC = succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate

IT = iminothiolane

CYTOTOXIC DRUGS:

MMAE = mono-methyl auristatin E (MW 718)

MMAF = variant of auristatin E (MMAE) with a phenylalanine at the C-terminus of the drug (MW 731.5)

MMAF-DMAEA = MMAF with DMAEA (dimethylaminoethylamine) in an amide linkage to the C-terminal phenylalanine (MW 801.5)

MMAF-TEG = MMAF with tetraethylene glycol esterified to the phenylalanine

MMAF-NtBu = N-t-butyl, attached as an amide to C-terminus of MMAF

DM1 = N(2')-deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine

DM3 = N(2')-deacetyl-N2-(4-mercapto-1-oxopentyl)-maytansine

DM4 = N(2')-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl)-maytansine

Further abbreviations are as follows: AE is auristatin E, Boc is N-(t-butoxycarbonyl), cit is citrulline, dap is dolaproine, DCC is 1,3-dicyclohexylcarbodiimide, DCM is dichloromethane, DEA is

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diethylamine, DEAD is diethylazodicarboxylate, DEPC is diethylphosphorylcyanidate, DIAD is diisopropylazodicarboxylate, DIEA is N,N-diisopropylethylamine, dil is dolaisoleucine, DMA is dimethylacetamide, DMAP is 4-dimethylaminopyridine, DME is ethyleneglycol dimethyl ether (or 1,2-dimethoxyethane), DMF is N,N-dimethylformamide, DMSO is dimethylsulfoxide, doe is dolaphenine, dov is N,N-dimethylvaline, DTNB is 5,5'-dithiobis(2-nitrobenzoic acid), DTPA is diethylenetriaminepentaacetic acid, DTT is dithiothreitol, EDCI is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EEDQ is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ES-MS is electrospray mass spectrometry, EtOAc is ethyl acetate, Fmoc is N-(9-fluorenylmethoxycarbonyl), gly is glycine, HATU is O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, HOBt is 1-hydroxybenzotriazole, HPLC is high pressure liquid chromatography, ile is isoleucine, lys is lysine, MeCN (CH_3CN) is acetonitrile, MeOH is methanol, Mtr is 4-anisyldiphenylmethyl (or 4-methoxytrityl),nor is (1S, 2R)-(+)-norephedrine, PBS is phosphate-buffered saline (pH 7.4), PEG is polyethylene glycol, Ph is phenyl, Pnp is p-nitrophenyl, MC is 6-maleimidocaproyl, phe is L-phenylalanine, PyBrop is bromo tris-pyrrolidino phosphonium hexafluorophosphate, SEC is size-exclusion chromatography, Su is succinimide, TFA is trifluoroacetic acid, TLC is thin layer chromatography, UV is ultraviolet, and val is valine.

COMPOSITIONS AND METHODS OF MAKING THE SAME

Antibodies that bind to STEAP-1 are provided. Immunoconjugates comprising anti-STEAP-1 antibodies are provided. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of disorders associated with altered expression, e.g., increased expression, of STEAP-1. In certain embodiments, antibodies or immunoconjugates of the invention are useful for the diagnosis or treatment of a cell proliferative disorder, such as cancer.

Anti-STEAP-1 Antibodies

In one aspect, the invention provides antibodies that bind to STEAP-1. In some embodiments, antibodies are provided that bind to a mature form of human and cynomolgus monkey (cyno) STEAP-1. In one such embodiment, a mature form of human STEAP-1 has an amino acid sequence of SEQ ID NO:1 (Figure 1). The cyno STEAP-1 has an amino acid sequence of SEQ ID NO:3 (Figure 1). In some embodiments, an antibody to STEAP-1 binds to a mature form of STEAP-1 expressed on the cell surface. In some embodiments, an antibody that binds to a mature form of STEAP-1 expressed on the cell surface inhibits the growth of the cell. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and inhibits cell proliferation. In certain embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the cell surface and induces cell death. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of STEAP-1 expressed on the surface of cancer cells. In some embodiments, an anti-STEAP-1 antibody binds to a mature form of

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STEAP-1 that is overexpressed on the surface of cancer cells relative to normal cells of the same tissue origin. In some embodiments, an anti-STEAP-1 antibody is conjugated to a cytotoxin or a detectable label and binds to STEAP-1 on a cell surface. In some embodiments, the antibody-toxin conjugate inhibits growth of the cell. In some embodiments, the antibody-detectable label conjugate causes a cell expressing STEAP-1 on its surface to be detectable in vitro or in vivo.

In one aspect, an anti-STEAP-1 antibody is a monoclonal antibody. In one aspect, an anti-STEAP-1 antibody is an antibody fragment, e.g., a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an anti-STEAP-1 antibody is a chimeric, humanized, or human antibody. In one aspect, any of the anti-STEAP-1 antibodies described herein are purified.

Exemplary monoclonal antibodies derived from a phage library are provided herein. The antigen used for screening the library was a polypeptide having the sequence of amino acid sequences of SEQ ID NO:28 or SEQ ID NO:30, corresponding to the extracellular domains (ECDs) of STEAP-1 beta and alpha. The antibodies resulting from the library screen are affinity matured.

In one aspect, monoclonal antibodies that compete with murine 120.545, 120 graft, and humanized 120v.24 binding to STEAP-1 are provided. Monoclonal antibodies that bind to the same epitope as murine 120.545, 120 graft, and humanized 120v.24 are also provided.

In one aspect of the invention, polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the invention, compositions comprising anti-STEAP-1 antibodies or polynucleotides encoding anti-STEAP-1 antibodies are provided. In certain embodiments, a composition of the invention is a pharmaceutical formulation for the treatment of a cell proliferative disorder, such as those enumerated herein.

A detailed description of exemplary anti-STEAP-1 antibodies is as follows:

1. Specific embodiments of anti-STEAP-1 antibodies

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain comprising SEQ ID NO:9 or 10 of Figure 2B. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising SEQ ID NO:6 of Figure 2A.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain comprising SEQ ID NO:9, having one or more of the following amino changes at the indicated Kabat position: A24V, V37I, V48M, F67I, and L78F. In one embodiment, the heavy chain comprises a heavy chain framework region selected from SEQ ID NOs:25, 75, 76, 77, 78, and 79. As used herein, heavy chain framework regions are designated "FR-H1-H4" or "HC-FR1-FR4," and light chain framework

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In one aspect, the invention provides an anti-STEAP-1 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from SEQ ID NOs:6, 11, 12, 13, 17, 18, 19, and 20. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a light chain variable domain comprising the amino acid sequence SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NO:9 and 10; and (b) a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence SEQ ID NO:6. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, additions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to STEAP-1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in the reference sequence, including but not limited to a sequence selected from SEQ ID NOs:9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, 79. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-STEAP-1 antibody comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:9 or 10 and a light chain variable domain comprising an amino acid sequence selected from SEQ ID NO:6.

In one aspect, the invention provides an anti-STEAP-1 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Figure 2B and/or (b) one, two, or three VL HVRs selected from those shown in Figure 2A. In one aspect, the invention provides an anti-STEAP-1 antibody comprising a heavy chain variable domain selected from those shown in Figure 2B and a light chain variable domain selected from those shown in Figure 2A.

2. Antibody Fragments

The present invention encompasses antibody fragments. Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the

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fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

3. Humanized Antibodies

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

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

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

4. Human Antibodies

Human anti-STEAP-1 antibodies of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s) as described above. Alternatively, human monoclonal anti-STEAP-1 antibodies of the invention can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain

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joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255 (1993); Bruggermann et al., Year in Immunol., 7: 33 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

5. Bispecific Antibodies

Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for STEAP-1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of STEAP-1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express STEAP-1. These antibodies possess a STEAP-1-binding arm and an arm which binds a cytotoxic agent, such as, e.g., saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305: 537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity

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chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published May 13, 1993, and in Traunecker et al., EMBO J., 10: 3655 (1991).

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

In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate

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antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

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

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

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

6. Multivalent Antibodies

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. In certain embodiments, the dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. In certain embodiments, a multivalent antibody comprises (or consists of) three to about eight antigen binding sites. In one such embodiment, a multivalent antibody comprises (or consists of) four antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (for example, two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-(X1)n -VD2-(X2)n -Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein may further comprise at least two (for example, four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

7. Single-Domain Antibodies

In some embodiments, an antibody of the invention is a single-domain antibody. A single-domain antibody is a single polyeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

8. Antibody Variants

In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological

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properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

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

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

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

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

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and US Patent No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)).

In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US 5739277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1,

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IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (US 2003/0190311, US6821505; US 6165745; US 5624821; US 5648260; US 6165745;US 5834 597).

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

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

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Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His(H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (e.g., improved) biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (e.g., the gene III product of M13) packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity). In order to identify candidate hypervariable region sites for modification, scanning mutagenesis (e.g., alanine scanning) can be performed to identify hypervariable

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region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to techniques known in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

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

It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions including that of a hinge cysteine.

In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the invention may comprise one or more alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001). Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1, WO99/51642. The contents of those patent publications are

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specifically incorporated herein by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

In one aspect, the invention provides antibodies comprising modifications in the interface of Fc polypeptides comprising the Fc region, wherein the modifications facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance is positionable in the cavity so as to promote complexing of the first and second Fc polypeptides. Methods of generating antibodies with these modifications are known in the art, e.g., as described in U.S. Pat. No. 5,731,168.

9. Antibody Derivatives

The antibodies of the present invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

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Certain Methods of Making Antibodies

1. Certain Hybridoma-Based Methods

The anti-STEAP-1 monoclonal antibodies of the invention can be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies to STEAP-1 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of STEAP-1 and an adjuvant. STEAP-1 may be prepared using methods well-known in the art, some of which are further described herein. For example, STEAP-1 may be produced recombinantly. In one embodiment, animals are immunized with a derivative of STEAP-1 that contains an extracellular portion of STEAP-1 fused to the Fc portion of an immunoglobulin heavy chain. In one embodiment, animals are immunized with an STEAP-1-IgG1 fusion protein. In one embodiment, animals are immunized with immunogenic derivatives of STEAP-1 in a solution with monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT), and the solution is injected intradermally at multiple sites. Two weeks later the animals are boosted. Seven to fourteen days later the animals are bled, and the serum is assayed for anti- STEAP-1 titer. Animals are boosted until titer plateaus.

Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

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

In certain embodiments, myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies

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(Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies that bind to STEAP-1. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

2. Certain Library Screening Methods

Anti-STEAP-1 antibodies of the invention can be made by using combinatorial libraries to screen for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are described generally in Hoogenboom et al. (2001) in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ), and in certain embodiments, in Lee et al. (2004) J. Mol. Biol. 340:1073-1093.

In principle, synthetic antibody clones are selected by screening phage libraries containing phage that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are panned by affinity chromatography against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen, and can be further enriched by additional cycles of antigen adsorption/elution. Any of the anti-STEAP-1 antibodies of the invention can be obtained by designing a suitable antigen screening procedure to select for the phage clone of interest followed by construction of a full length anti-STEAP-1 antibody clone using the Fv sequences from the phage clone of interest and suitable constant region (Fc) sequences described in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

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In certain embodiments, the antigen-binding domain of an antibody is formed from two variable (V) regions of about 110 amino acids, one each from the light (VL) and heavy (VH) chains, that both present three hypervariable loops (HVRs) or complementarity-determining regions (CDRs). Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). As used herein, scFv encoding phage clones and Fab encoding phage clones are collectively referred to as "Fv phage clones" or "Fv clones."

Repertoires of VH and VL genes can be separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned to provide a single source of human antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

In certain embodiments, filamentous phage is used to display antibody fragments by fusion to the minor coat protein pIII. The antibody fragments can be displayed as single chain Fv fragments, in which VH and VL domains are connected on the same polypeptide chain by a flexible polypeptide spacer, e.g. as described by Marks et al., J. Mol. Biol., 222: 581-597 (1991), or as Fab fragments, in which one chain is fused to pIII and the other is secreted into the bacterial host cell periplasm where assembly of a Fab-coat protein structure which becomes displayed on the phage surface by displacing some of the wild type coat proteins, e.g. as described in Hoogenboom et al., Nucl. Acids Res., 19: 4133-4137 (1991).

In general, nucleic acids encoding antibody gene fragments are obtained from immune cells harvested from humans or animals. If a library biased in favor of anti-STEAP-1 clones is desired, the subject is immunized with STEAP-1 to generate an antibody response, and spleen cells and/or circulating B cells other peripheral blood lymphocytes (PBLs) are recovered for library construction. In a preferred embodiment, a human antibody gene fragment library biased in favor of anti-STEAP-1 clones is obtained by generating an anti-STEAP-1 antibody response in transgenic mice carrying a functional human immunoglobulin gene array (and lacking a functional endogenous antibody production system) such that STEAP-1 immunization gives rise to B cells producing human antibodies against STEAP-1. The generation of human antibody-producing transgenic mice is described below.

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Additional enrichment for anti-STEAP-1 reactive cell populations can be obtained by using a suitable screening procedure to isolate B cells expressing STEAP-1-specific membrane bound antibody, e.g., by cell separation using STEAP-1 affinity chromatography or adsorption of cells to fluorochrome-labeled STEAP-1 followed by flow-activated cell sorting (FACS).

Alternatively, the use of spleen cells and/or B cells or other PBLs from an unimmunized donor provides a better representation of the possible antibody repertoire, and also permits the construction of an antibody library using any animal (human or non-human) species in which STEAP-1 is not antigenic. For libraries incorporating in vitro antibody gene construction, stem cells are harvested from the subject to provide nucleic acids encoding unrearranged antibody gene segments. The immune cells of interest can be obtained from a variety of animal species, such as human, mouse, rat, lagomorpha, luprine, canine, feline, porcine, bovine, equine, and avian species, etc.

Nucleic acid encoding antibody variable gene segments (including VH and VL segments) are recovered from the cells of interest and amplified. In the case of rearranged VH and VL gene libraries, the desired DNA can be obtained by isolating genomic DNA or mRNA from lymphocytes followed by polymerase chain reaction (PCR) with primers matching the 5' and 3' ends of rearranged VH and VL genes as described in Orlandi et al., Proc. Natl. Acad. Sci. (USA), 86: 3833-3837 (1989), thereby making diverse V gene repertoires for expression. The V genes can be amplified from cDNA and genomic DNA, with back primers at the 5' end of the exon encoding the mature V-domain and forward primers based within the J-segment as described in Orlandi et al. (1989) and in Ward et al., Nature, 341: 544-546 (1989). However, for amplifying from cDNA, back primers can also be based in the leader exon as described in Jones et al., Biotechnol., 9: 88-89 (1991), and forward primers within the constant region as described in Sastry et al., Proc. Natl. Acad. Sci. (USA), 86: 5728-5732 (1989). To maximize complementarity, degeneracy can be incorporated in the primers as described in Orlandi et al. (1989) or Sastry et al. (1989). In certain embodiments, library diversity is maximized by using PCR primers targeted to each V-gene family in order to amplify all available VH and VL arrangements present in the immune cell nucleic acid sample, e.g. as described in the method of Marks et al., J. Mol. Biol., 222: 581-597 (1991) or as described in the method of Orum et al., Nucleic Acids Res., 21: 4491-4498 (1993). For cloning of the amplified DNA into expression vectors, rare restriction sites can be introduced within the PCR primer as a tag at one end as described in Orlandi et al. (1989), or by further PCR amplification with a tagged primer as described in Clackson et al., Nature, 352: 624-628 (1991).

Repertoires of synthetically rearranged V genes can be derived in vitro from V gene segments. Most of the human VH-gene segments have been cloned and sequenced (reported in Tomlinson et al., J. Mol. Biol., 227: 776-798 (1992)), and mapped (reported in Matsuda et al., Nature Genet., 3: 88-94 (1993); these cloned segments (including all the major conformations of the H1 and H2 loop) can be used to

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generate diverse VH gene repertoires with PCR primers encoding H3 loops of diverse sequence and length as described in Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). VH repertoires can also be made with all the sequence diversity focused in a long H3 loop of a single length as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 89: 4457-4461 (1992). Human Vκ and Vλ segments have been cloned and sequenced (reported in Williams and Winter, Eur. J. Immunol., 23: 1456-1461 (1993)) and can be used to make synthetic light chain repertoires. Synthetic V gene repertoires, based on a range of VH and VL folds, and L3 and H3 lengths, will encode antibodies of considerable structural diversity. Following amplification of V-gene encoding DNAs, germline V-gene segments can be rearranged in vitro according to the methods of Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992).

Repertoires of antibody fragments can be constructed by combining VH and VL gene repertoires together in several ways. Each repertoire can be created in different vectors, and the vectors recombined in vitro, e.g., as described in Hogrefe et al., Gene, 128: 119-126 (1993), or in vivo by combinatorial infection, e.g., the loxP system described in Waterhouse et al., Nucl. Acids Res., 21: 2265-2266 (1993). The in vivo recombination approach exploits the two-chain nature of Fab fragments to overcome the limit on library size imposed by E. coli transformation efficiency. Naive VH and VL repertoires are cloned separately, one into a phagemid and the other into a phage vector. The two libraries are then combined by phage infection of phagemid-containing bacteria so that each cell contains a different combination and the library size is limited only by the number of cells present (about 10^{12} clones). Both vectors contain in vivo recombination signals so that the VH and VL genes are recombined onto a single replicon and are co-packaged into phage virions. These huge libraries provide large numbers of diverse antibodies of good affinity (K_d^{-1} of about 10^{-8} M).

Alternatively, the repertoires may be cloned sequentially into the same vector, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci. USA, 88: 7978-7982 (1991), or assembled together by PCR and then cloned, e.g. as described in Clackson et al., Nature, 352: 624-628 (1991). PCR assembly can also be used to join VH and VL DNAs with DNA encoding a flexible peptide spacer to form single chain Fv (scFv) repertoires. In yet another technique, "in cell PCR assembly" is used to combine VH and VL genes within lymphocytes by PCR and then clone repertoires of linked genes as described in Embleton et al., Nucl. Acids Res., 20: 3831-3837 (1992).

The antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_d^{-1} of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked in vitro by constructing and reselecting from secondary libraries as described in Winter et al. (1994), supra. For example, mutation can be introduced at random in vitro by using error-prone polymerase (reported in Leung et al., Technique, 1: 11-15 (1989)) in the method of Hawkins et al., J. Mol. Biol., 226: 889-896 (1992) or in the method of Gram et al., Proc. Natl. Acad. Sci USA, 89: 3576-3580 (1992). Additionally, affinity maturation can be

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performed by randomly mutating one or more CDRs, e.g. using PCR with primers carrying random sequence spanning the CDR of interest, in selected individual Fv clones and screening for higher affinity clones. WO 9607754 (published 14 March 1996) described a method for inducing mutagenesis in a complementarity determining region of an immunoglobulin light chain to create a library of light chain genes. Another effective approach is to recombine the VH or VL domains selected by phage display with repertoires of naturally occurring V domain variants obtained from unimmunized donors and screen for higher affinity in several rounds of chain reshuffling as described in Marks et al., Biotechnol., 10: 779-783 (1992). This technique allows the production of antibodies and antibody fragments with affinities of about 10^{-9} M or less.

Screening of the libraries can be accomplished by various techniques known in the art. For example, STEAP-1 can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, or conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning phage display libraries.

The phage library samples are contacted with immobilized STEAP-1 under conditions suitable for binding at least a portion of the phage particles with the adsorbent. Normally, the conditions, including pH, ionic strength, temperature and the like are selected to mimic physiological conditions. The phages bound to the solid phase are washed and then eluted by acid, e.g. as described in Barbas et al., Proc. Natl. Acad. Sci USA, 88: 7978-7982 (1991), or by alkali, e.g. as described in Marks et al., J. Mol. Biol., 222: 581-597 (1991), or by STEAP-1 antigen competition, e.g. in a procedure similar to the antigen competition method of Clackson et al., Nature, 352: 624-628 (1991). Phages can be enriched 20-1,000-fold in a single round of selection. Moreover, the enriched phages can be grown in bacterial culture and subjected to further rounds of selection.

The efficiency of selection depends on many factors, including the kinetics of dissociation during washing, and whether multiple antibody fragments on a single phage can simultaneously engage with antigen. Antibodies with fast dissociation kinetics (and weak binding affinities) can be retained by use of short washes, multivalent phage display and high coating density of antigen in solid phase. The high density not only stabilizes the phage through multivalent interactions, but favors rebinding of phage that has dissociated. The selection of antibodies with slow dissociation kinetics (and good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass et al., Proteins, 8: 309-314 (1990) and in WO 92/09690, and a low coating density of antigen as described in Marks et al., Biotechnol., 10: 779-783 (1992).

It is possible to select between phage antibodies of different affinities, even with affinities that differ slightly, for STEAP-1. However, random mutation of a selected antibody (e.g. as performed in some affinity maturation techniques) is likely to give rise to many mutants, most binding to antigen, and a few

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with higher affinity. With limiting STEAP-1, rare high affinity phage could be competed out. To retain all higher affinity mutants, phages can be incubated with excess biotinylated STEAP-1, but with the biotinylated STEAP-1 at a concentration of lower molarity than the target molar affinity constant for STEAP-1. The high affinity-binding phages can then be captured by streptavidin-coated paramagnetic beads. Such "equilibrium capture" allows the antibodies to be selected according to their affinities of binding, with sensitivity that permits isolation of mutant clones with as little as two-fold higher affinity from a great excess of phages with lower affinity. Conditions used in washing phages bound to a solid phase can also be manipulated to discriminate on the basis of dissociation kinetics.

Anti-STEAP-1 clones may be selected based on activity. In certain embodiments, the invention provides anti-STEAP-1 antibodies that bind to living cells that naturally express STEAP-1. In one embodiment, the invention provides anti-STEAP-1 antibodies that block the binding between a STEAP-1 ligand and STEAP-1, but do not block the binding between a STEAP-1 ligand and a second protein. Fv clones corresponding to such anti-STEAP-1 antibodies can be selected by (1) isolating anti-STEAP-1 clones from a phage library as described above, and optionally amplifying the isolated population of phage clones by growing up the population in a suitable bacterial host; (2) selecting STEAP-1 and a second protein against which blocking and non-blocking activity, respectively, is desired; (3) adsorbing the anti-STEAP-1 phage clones to immobilized STEAP-1; (4) using an excess of the second protein to elute any undesired clones that recognize STEAP-1-binding determinants which overlap or are shared with the binding determinants of the second protein; and (5) eluting the clones which remain adsorbed following step (4). Optionally, clones with the desired blocking/non-blocking properties can be further enriched by repeating the selection procedures described herein one or more times.

DNA encoding hybridoma-derived monoclonal antibodies or phage display Fv clones of the invention is readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide primers designed to specifically amplify the heavy and light chain coding regions of interest from hybridoma or phage DNA template). Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of the desired monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs, 130: 151 (1992).

DNA encoding the Fv clones of the invention can be combined with known DNA sequences encoding heavy chain and/or light chain constant regions (e.g. the appropriate DNA sequences can be obtained from Kabat et al., supra) to form clones encoding full or partial length heavy and/or light chains. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM,

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IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. An Fv clone derived from the variable domain DNA of one animal (such as human) species and then fused to constant region DNA of another animal species to form coding sequence(s) for "hybrid," full length heavy chain and/or light chain is included in the definition of "chimeric" and "hybrid" antibody as used herein. In certain embodiments, an Fv clone derived from human variable DNA is fused to human constant region DNA to form coding sequence(s) for full- or partial-length human heavy and/or light chains.

DNA encoding anti-STEAP-1 antibody derived from a hybridoma of the invention can also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of homologous murine sequences derived from the hybridoma clone (e.g. as in the method of Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). DNA encoding a hybridoma- or Fv clone-derived antibody or fragment can be further modified by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In this manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of the Fv clone or hybridoma clone-derived antibodies of the invention.

3. Vectors, Host Cells, and Recombinant Methods

For recombinant production of an antibody of the invention, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating antibodies using prokaryotic host cells:

Vector Construction

Polynucleotide sequences encoding polypeptide components of the antibody of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for

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the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically transformed using pBR322, a plasmid derived from an E. coli species. pBR322 contains genes encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Patent No. 5,648,237.

In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λGEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as E. coli LE392.

The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

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Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β-galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

In another aspect, the production of the immunoglobulins according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB- strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

Antibodies of the invention can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number

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or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a "codon bank" at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) METHODS: A Companion to Methods in Enzymol. 4:151-158.

In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5, 840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the invention.

Prokaryotic host cells suitable for expressing antibodies of the invention include Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., E. coli), Bacilli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the invention. Examples of E. coli strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli B, E. coliλ 1776 (ATCC 31,537) and E. coli RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

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Antibody Production

Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

Prokaryotic cells used to produce the polypeptides of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for E. coli growth, growth temperatures range from about 20°C to about 39°C; from about 25°C to about 37°C; or about 30°C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for E. coli, the pH is from about 6.8 to about 7.4, or about 7.0.

If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al., J. Immunol. Methods (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

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In one embodiment, the expressed polypeptides of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

In one aspect of the invention, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Patent No. 6,083,715; Georgiou et al., U.S. Patent No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present

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invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Patent No. 5,264,365; Georgiou et al., U.S. Patent No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

In one embodiment, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

Antibody Purification

In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the invention. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating antibodies using eukaryotic host cells:

A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

Signal sequence component

A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a

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signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

Origin of replication

Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

Selection gene component

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

Promoter component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (e.g., an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region

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located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982), describing expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

Enhancer element component

Transcription of DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody polypeptide-encoding sequence, but is generally located at a site 5' from the promoter.

Transcription termination component

Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the

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mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

Selection and transformation of host cells

Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)) ; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)) ; mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Culturing the host cells

The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

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Purification of antibody

When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Immunoconjugates

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The invention also provides immunoconjugates (interchangeably referred to as "antibody-drug conjugates," or "ADCs") comprising any of the anti-STEAP-1 antibodies of the invention conjugated to one or more cytotoxic agents, such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and a chemotherapeutic agent or other toxin. Chemotherapeutic agents useful in the generation of immunoconjugates are described herein (e.g., above). Enzymatically active toxins and fragments thereof can also be used and are described herein.

In certain embodiments, an immunoconjugate comprises an anti-STEAP-1 antibody and one or more small molecule toxins, including, but not limited to, small molecule drugs such as a calicheamicin, maytansinoid, dolastatin, auristatin, trichothecene, and CC1065, and the derivatives of these drugs that have cytotoxic activity. Examples of such immunoconjugates are discussed in further detail below.

1. Exemplary Immunoconjugates

An immunoconjugate (or "antibody-drug conjugate" ("ADC")) of the invention may be of Formula I, below, wherein an anti-STEAP-1 antibody is conjugated (i.e., covalently attached) to one or more drug moieties (D) through an optional linker (L).

$$Ab\text{–}(L\text{–}D)_p \qquad \text{Formula I}$$

Accordingly, the anti-STEAP-1 antibody may be conjugated to the drug either directly or via a linker. In Formula I, p is the average number of drug moieties per antibody, which can range, e.g., from about 1 to about 20 drug moieties per antibody, and in certain embodiments, from 1 to about 8 drug moieties per antibody.

Exemplary Linkers

Exemplary linkers and drug moieties are disclosed herein. A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"), and ethyleneoxy $-CH_2CH_2O-$ as one or more repeating units ("EO" or "PEO"). Various linker components are known in the art, some of which are described below.

A linker may be a "cleavable linker," facilitating release of a drug in the cell. For example, an acid-labile linker (e.g., hydrazone), protease-sensitive (e.g., peptidase-sensitive) linker, photolabile linker,

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dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

In one embodiment, linker L of an ADC has the formula:

$$-A_a-W_w-Y_y-$$

wherein:

-A- is a Stretcher unit covalently attached to a cysteine thiol of the antibody (Ab);

a is 0 or 1;

each -W- is independently an Amino Acid unit;

w is independently an integer ranging from 0 to 12;

-Y- is a Spacer unit covalently attached to the drug moiety; and

y is 0, 1 or 2.

Stretcher unit

The Stretcher unit (-A-), when present, is capable of linking an antibody unit to an amino acid unit (-W-). In this regard an antibody (Ab) has a free cysteine thiol group that can form a bond with an electrophilic functional group of a Stretcher Unit. Exemplary stretcher units in Formula **I** conjugates are depicted by Formulas **II** and **III**, wherein Ab-, -W-, -Y-, -D, w and y are as defined above, and R^{17} is a divalent radical selected from $(CH_2)_r$, C_3-C_8 carbocyclyl, O–$(CH_2)_r$, arylene, $(CH_2)_r$–arylene, –arylene–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbocyclyl), (C_3-C_8 carbocyclyl)–$(CH_2)_r$, C_3-C_8 heterocyclyl, $(CH_2)_r$–(C_3-C_8 heterocyclyl), –(C_3-C_8 heterocyclyl)–$(CH_2)_r$–, $-(CH_2)_rC(O)NR^b(CH_2)_r-$, $-(CH_2CH_2O)_r-$, $-(CH_2CH_2O)_r-CH_2-$, $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-$, $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$, $-(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r-$, $-(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$, and $-(CH_2CH_2O)_rC(O)NR^b(CH_2)_r-$; where R^b is H, C_1-C_6 alkyl, phenyl, or benzyl; and r is independently an integer ranging from 1-10.

Arylene includes divalent aromatic hydrocarbon radicals of 6-20 carbon atoms derived by the removal of two hydrogen atoms from the aromatic ring system. Typical arylene groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

Heterocyclyl groups include a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo

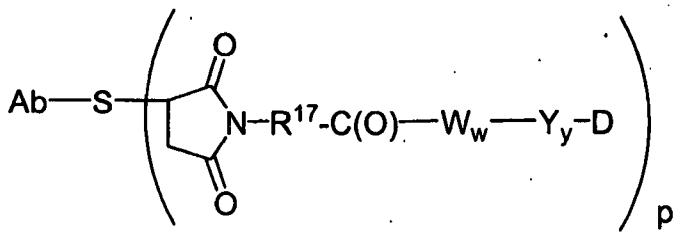
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[4,5], [5,5], [5,6], or [6,6] system. Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and J. Am. Chem. Soc. (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4Ah-carbazolyl, carbazolyl, β-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

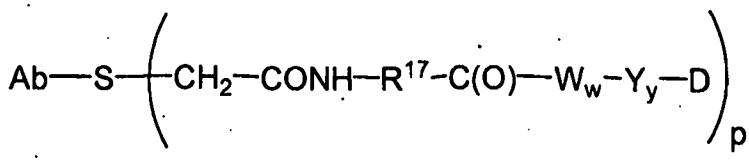
Carbocyclyl groups include a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

It is to be understood from all the exemplary embodiments of Formula **I** ADC such as **II-VI**, that even where not denoted expressly, from 1 to 4 drug moieties are linked to an antibody (p = 1-4), depending on the number of engineered cysteine residues.



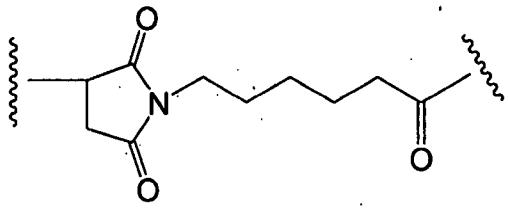
II

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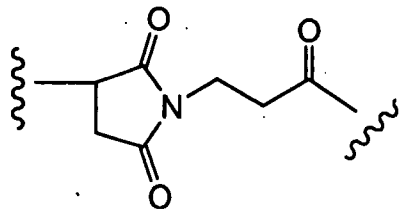
III

An illustrative Formula **II** Stretcher unit is derived from maleimido-caproyl (MC) wherein R^{17} is -$(CH_2)_5$-:



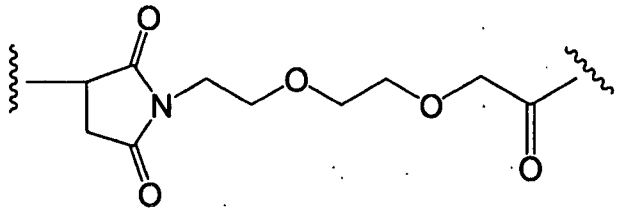
MC

An illustrative Stretcher unit of Formula **II**, and is derived from maleimido-propanoyl (MP) wherein R^{17} is -$(CH_2)_2$-:

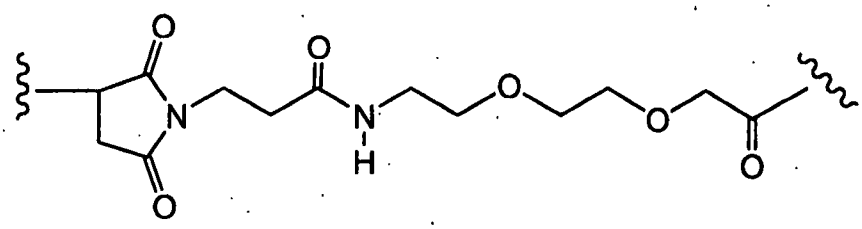


MP

Another illustrative Stretcher unit of Formula **II** wherein R^{17} is -$(CH_2CH_2O)_r$-CH_2 - and r is 2:

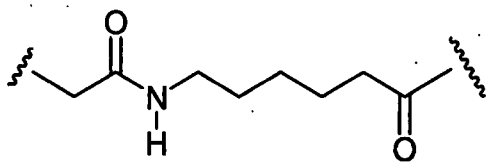


Another illustrative Stretcher unit of Formula **II** wherein R^{17} is –$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$–$CH_2$– where R^b is H and each r is 2:



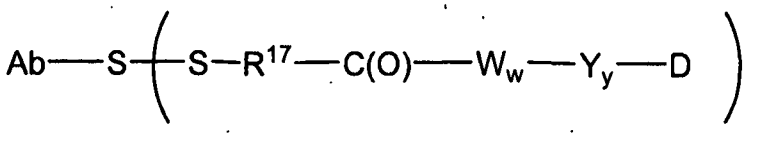
MPEG

An illustrative Stretcher unit of Formula **III** wherein R^{17} is -$(CH_2)_5$-:



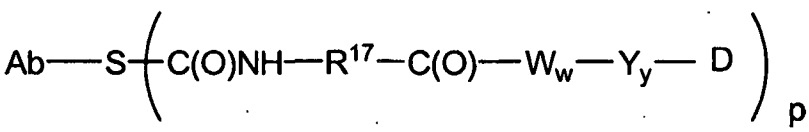
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In another embodiment, the Stretcher unit is linked to the cysteine engineered anti-____ antibody via a disulfide bond between the engineered cystein sulfur atom of the antibody and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted by Formula **IV**, wherein R^{17}, Ab-, -W-, -Y-, -D, w and y are as defined above.

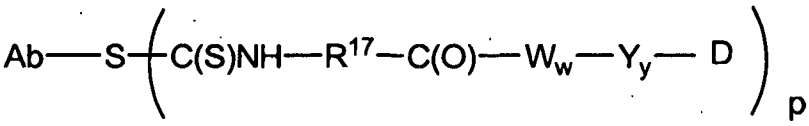


IV

In yet another embodiment, the reactive group of the Stretcher contains a thiol-reactive functional group that can form a bond with a free cysteine thiol of an antibody. Examples of thiol-reaction functional groups include, but are not limited to, maleimide, α-haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. Representative Stretcher units of this embodiment are depicted by Formulas **Va** and **Vb**, wherein $-R^{17}-$, Ab-, -W-, -Y-, -D, w and y are as defined above;



Va



Vb

In another embodiment, the linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to an antibody (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768; King (2002) Tetrahedron Letters 43:1987-1990). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

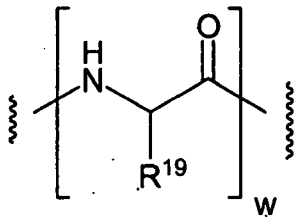
Amino acid unit

The linker may comprise amino acid residues. The Amino Acid unit ($-W_w-$), when present, links the antibody (Ab) to the drug moiety (D) of the cysteine engineered antibody-drug conjugate (ADC) of the invention.

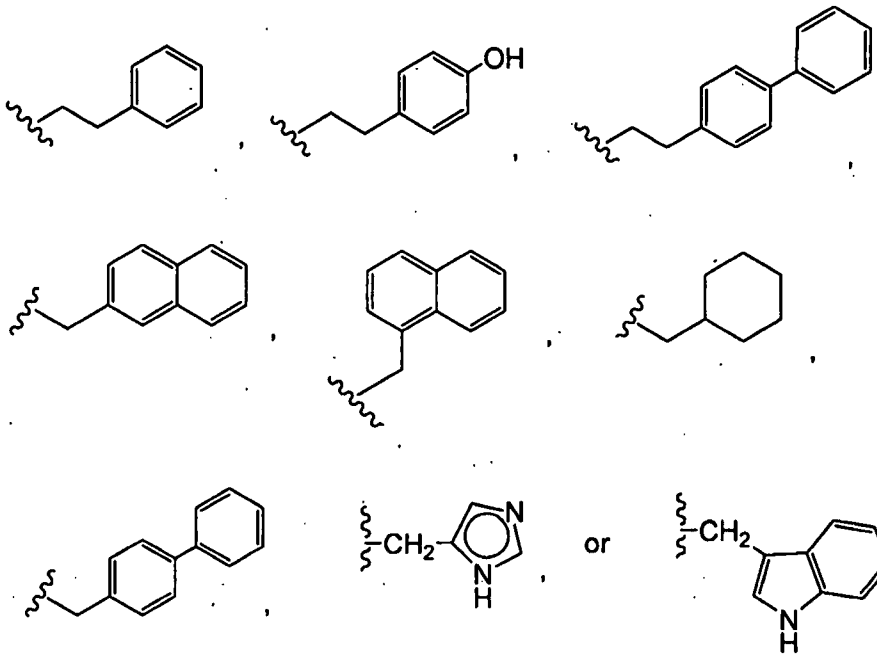
$-W_w-$ is a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Amino acid residues which comprise the Amino Acid unit include those occurring naturally, as well as minor amino acids and non-naturally

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occurring amino acid analogs, such as citrulline. Each -W- unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 to 12:



wherein R^{19} is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, -CH_2OH, -$CH(OH)CH_3$, -$CH_2CH_2SCH_3$, -CH_2CONH_2, -CH_2COOH, -$CH_2CH_2CONH_2$, -CH_2CH_2COOH, -$(CH_2)_3NHC(=NH)NH_2$, -$(CH_2)_3NH_2$, -$(CH_2)_3NHCOCH_3$, -$(CH_2)_3NHCHO$, -$(CH_2)_4NHC(=NH)NH_2$, -$(CH_2)_4NH_2$, -$(CH_2)_4NHCOCH_3$, -$(CH_2)_4NHCHO$, -$(CH_2)_3NHCONH_2$, -$(CH_2)_4NHCONH_2$, -$CH_2CH_2CH(OH)CH_2NH_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,



When R^{19} is other than hydrogen, the carbon atom to which R^{19} is attached is chiral. Each carbon atom to which R^{19} is attached is independently in the (S) or (R) configuration, or a racemic mixture. Amino acid units may thus be enantiomerically pure, racemic, or diastereomeric.

Exemplary $-W_w-$ Amino Acid units include a dipeptide, a tripeptide, a tetrapeptide or a pentapeptide. Exemplary dipeptides include: valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe). Exemplary tripeptides include: glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). Amino acid residues which comprise an amino acid linker component include those occurring naturally, as well as minor amino acids and non-naturally occurring amino acid analogs, such as citrulline.

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The Amino Acid unit can be enzymatically cleaved by one or more enzymes, including a tumor-associated protease, to liberate the Drug moiety (-D), which in one embodiment is protonated in vivo upon release to provide a Drug (D). Amino acid linker components can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Spacer unit

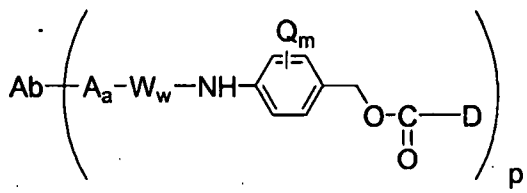
The Spacer unit ($-Y_y-$), when present (y = 1 or 2), links an Amino Acid unit ($-W_w-$) to the drug moiety (D) when an Amino Acid unit is present (w = 1-12). Alternately, the Spacer unit links the Stretcher unit to the Drug moiety when the Amino Acid unit is absent. The Spacer unit also links the drug moiety to the antibody unit when both the Amino Acid unit and Stretcher unit are absent (w, y = 0). Spacer units are of two general types: self-immolative and non self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to the Drug moiety after cleavage, particularly enzymatic, of an Amino Acid unit from the antibody-drug conjugate or the Drug moiety-linker. When an ADC containing a glycine-glycine Spacer unit or a glycine Spacer unit undergoes enzymatic cleavage via a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease, a glycine-glycine-Drug moiety or a glycine-Drug moiety is cleaved from Ab-A_a-Ww-. In one embodiment, an independent hydrolysis reaction takes place within the target cell, cleaving the glycine-Drug moiety bond and liberating the Drug.

In another embodiment, -Y_y- is a p-aminobenzylcarbamoyl (PAB) unit whose phenylene portion is substituted with Q_m wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen,- nitro or -cyano; and m is an integer ranging from 0-4.

Exemplary embodiments of a non self-immolative Spacer unit (-Y-) are: -Gly-Gly- ; -Gly- ; -Ala-Phe- ; -Val-Cit- .

In one embodiment, a Drug moiety-linker or an ADC is provided in which the Spacer unit is absent (y=0), or a pharmaceutically acceptable salt or solvate thereof.

Alternatively, an ADC containing a self-immolative Spacer unit can release -D. In one embodiment, -Y- is a PAB group that is linked to -W_w- via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group, where the ADC has the exemplary structure:

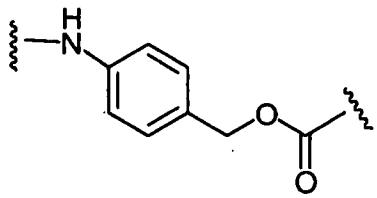


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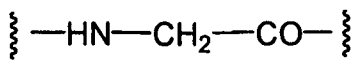
wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to 4.

Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237), heterocyclic PAB analogs (US 2005/0256030), beta-glucuronide (WO 2007/011968), and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al (1995) Chemistry Biology 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al (1972) J. Amer. Chem. Soc. 94:5815) and 2-aminophenylpropionic acid amides (Amsberry, et al (1990) J. Org. Chem. 55:5867). Elimination of amine-containing drugs that are substituted at glycine (Kingsbury et al (1984) J. Med. Chem. 27:1447) are also examples of self-immolative spacer useful in ADCs.

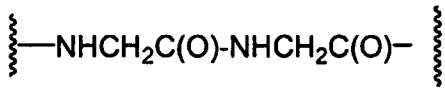
Exemplary Spacer units (-Y_y-) are represented by Formulas **X-XII**:



X



XI



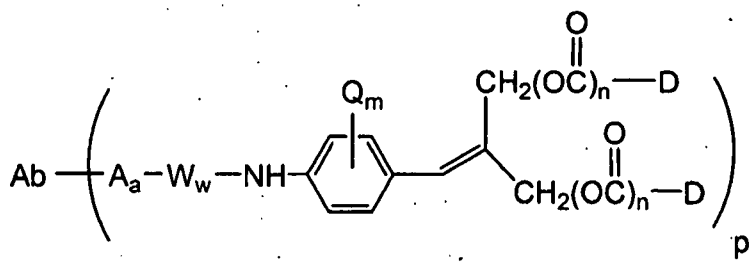
XII

Dendritic linkers

In another embodiment, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to an antibody (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker. Exemplary embodiments of branched, dendritic linkers include 2,6-bis(hydroxymethyl)-p-cresol and 2,4,6-tris(hydroxymethyl)-phenol dendrimer units (WO 2004/01993; Szalai et al (2003) J. Amer. Chem. Soc. 125:15688-15689; Shamis et al (2004) J. Amer. Chem. Soc. 126:1726-1731; Amir et al (2003) Angew. Chem. Int. Ed. 42:4494-4499).

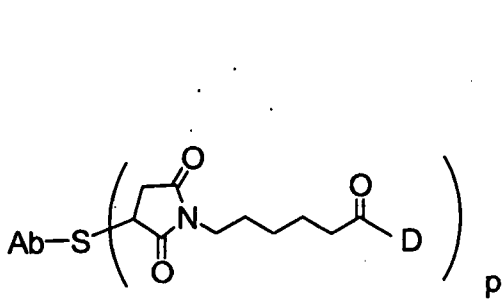
In one embodiment, the Spacer unit is a branched bis(hydroxymethyl)styrene (BHMS), which can be used to incorporate and release multiple drugs, having the structure:

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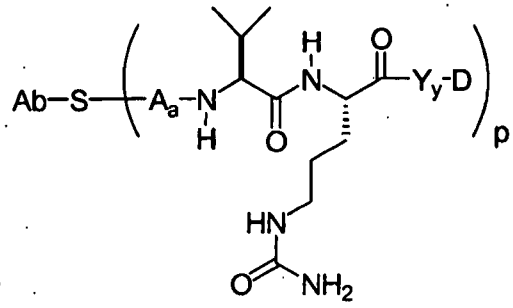


comprising a 2-(4-aminobenzylidene)propane-1,3-diol dendrimer unit (WO 2004/043493; de Groot et al (2003) Angew. Chem. Int. Ed. 42:4490-4494), wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges ranging from 1 to 4.

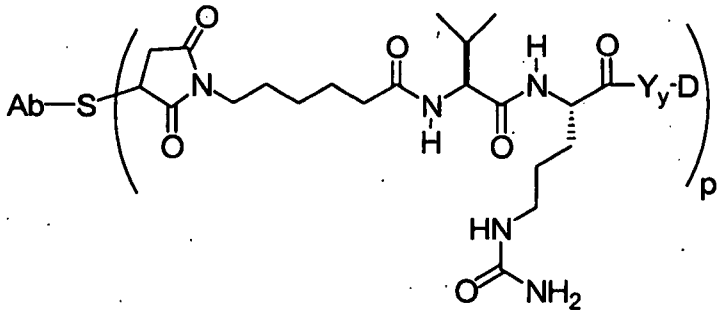
Exemplary embodiments of the Formula **I** antibody-drug conjugate compounds include **XIIIa** (MC), **XIIIb** (val-cit), **XIIIc** (MC-val-cit), and **XIIId** (MC-val-cit-PAB):



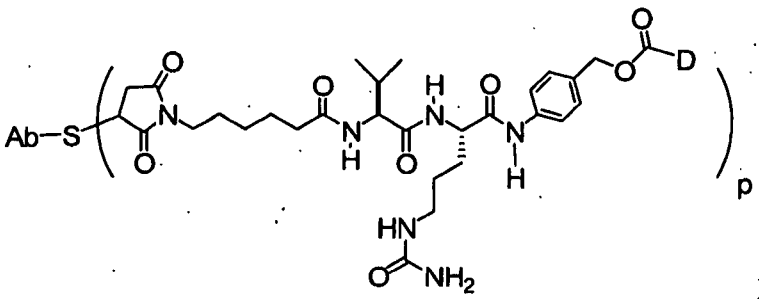
XIIIa



XIIIb



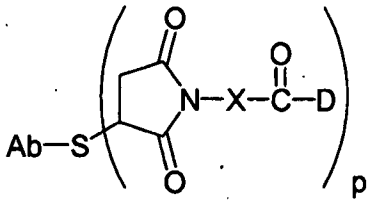
XIIIc



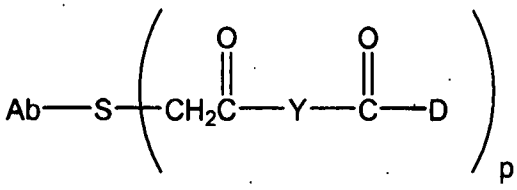
XIIId

Other exemplary embodiments of the Formula **Ia** antibody-drug conjugate compounds include **XIVa-e**:

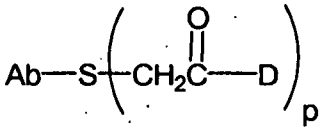
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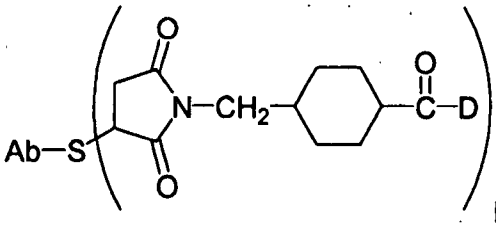
XIVa



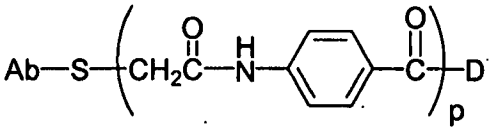
XIVb



XIVc

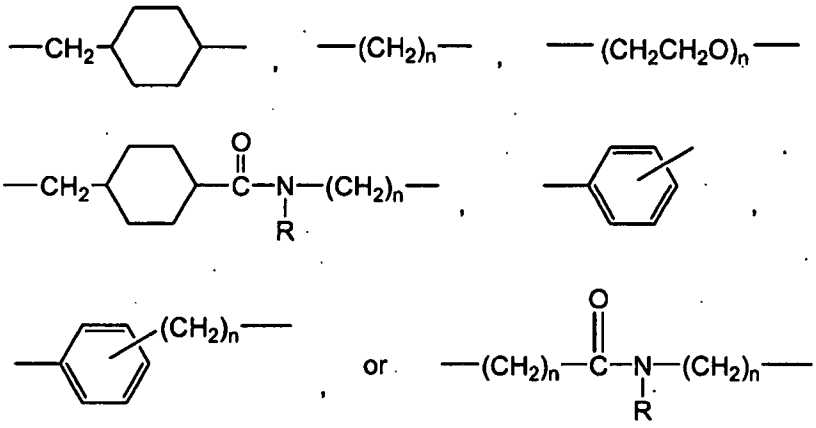


XIVd



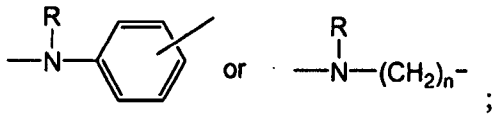
XIVe

where X is:



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Y is:



and R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

In another embodiment, a Linker has a reactive functional group which has a nucleophilic group that is reactive to an electrophilic group present on an antibody. Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a Linker include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a Linker.

Typically, peptide-type Linkers 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 (E. Schröder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press) which is well known in the field of peptide chemistry. Linker intermediates may be assembled with any combination or sequence of reactions including Spacer, Stretcher, and Amino Acid units. The Spacer, Stretcher, and Amino Acid units may employ reactive functional groups which are electrophilic, nucleophilic, or free radical in nature. Reactive functional groups include, but are not limited to carboxyls, hydroxyls, para-nitrophenylcarbonate, isothiocyanate, and leaving groups, such as O-mesyl, O-tosyl, -Cl, -Br, -I; or maleimide.

In another embodiment, the Linker may be substituted with groups which modulated solubility or reactivity. For example, a charged substituent such as sulfonate ($-SO_3^-$) or ammonium, may increase water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC.

Exemplary Drug Moieties

Maytansine and maytansinoids

In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to one or more maytansinoid molecules. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and

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derivatives and analogues thereof are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

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 or produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973). Maytansinol and maytansinol analogues may also be prepared synthetically according to known methods.

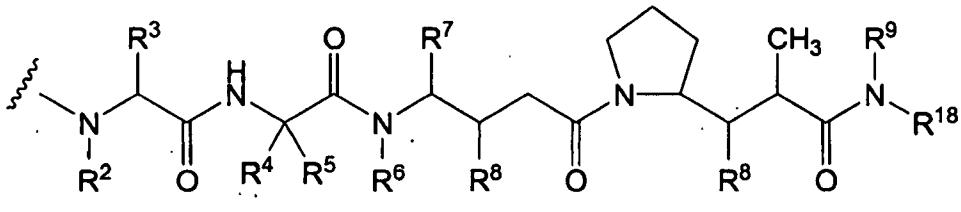
Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, as disclosed herein.

Auristatins and dolastatins

In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to dolastatin or a dolastatin peptidic analog or derivative, e.g., an auristatin (US Pat. Nos. 5635483; 5780588). 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 (US Pat. No.5663149) 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).

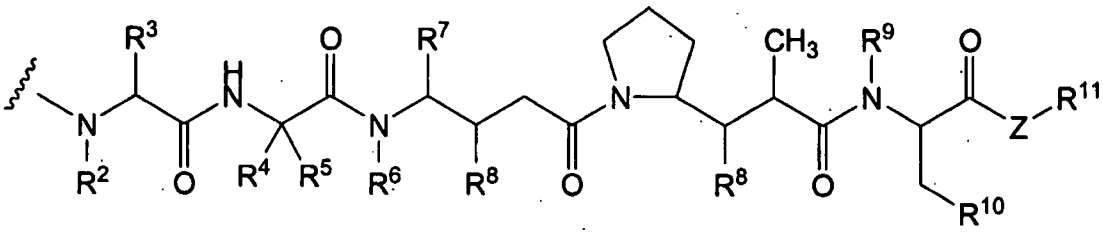
Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004, the disclosure of which is expressly incorporated by reference in its entirety.

A peptidic drug moiety may be selected from Formulas D_E and D_F below:



D_E

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D_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8 heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O-(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;

each occurrence of R^{16} is independently H, C_1-C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2-$aryl, $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

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In one embodiment, R^3, R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is –H or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is -H, and R^7 is sec-butyl.

In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is -H.

In still another embodiment, each occurrence of R^8 is $-OCH_3$.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is -H, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is -H.

In one embodiment, Z is -O- or -NH-.

In one embodiment, R^{10} is aryl.

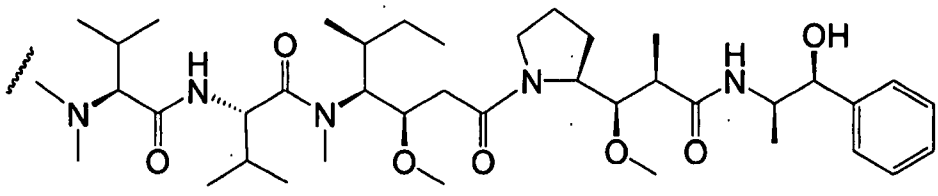
In an exemplary embodiment, R^{10} is -phenyl.

In an exemplary embodiment, when Z is -O-, R^{11} is –H, methyl or t-butyl.

In one embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is C_1-C_8 alkyl or $-(CH_2)_n-COOH$.

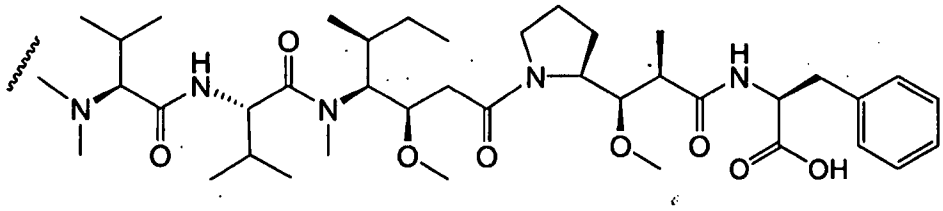
In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



MMAE

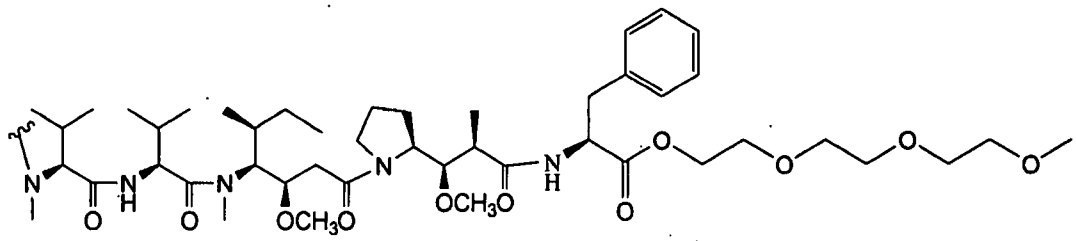
An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate (*see* US 2005/0238649 and Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124):

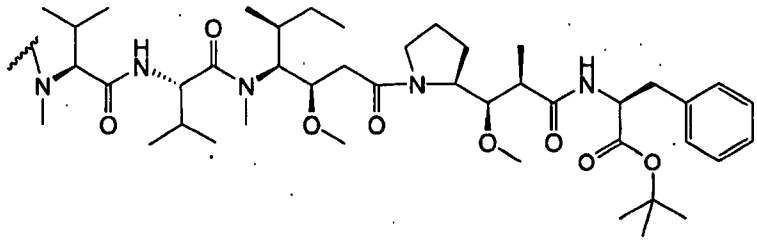


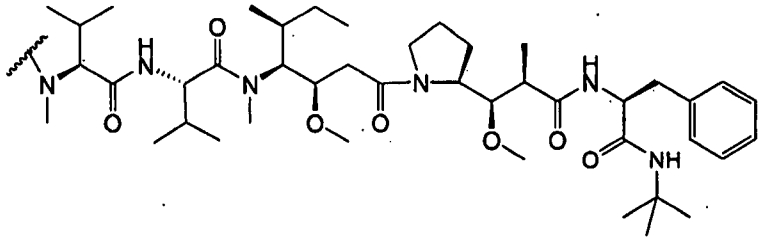
MMAF

Other drug moieties include the following MMAF derivatives, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

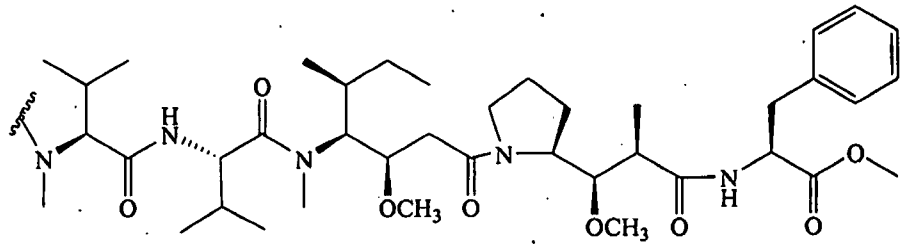
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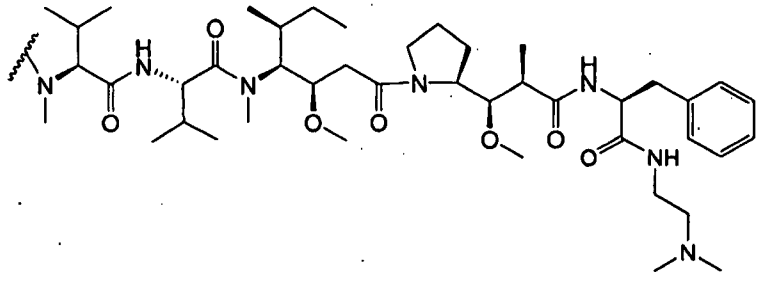




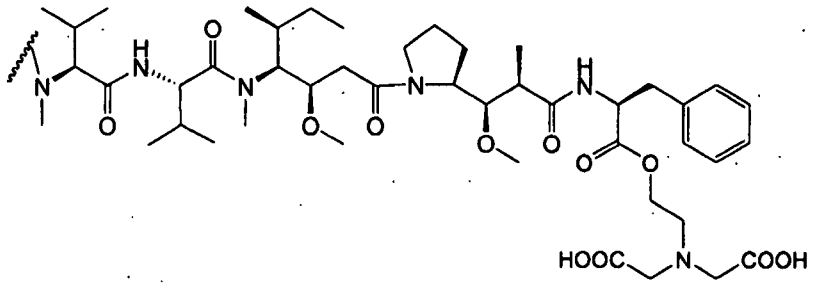


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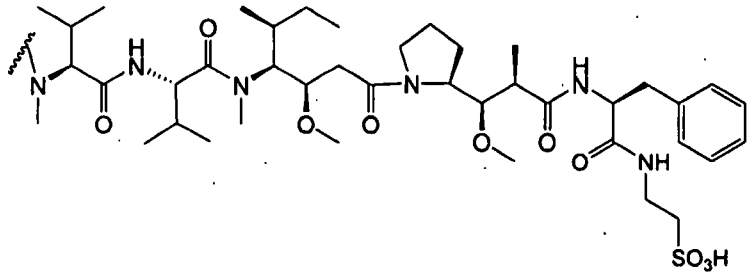




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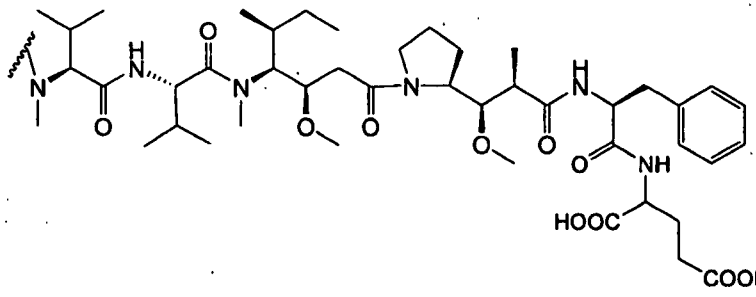


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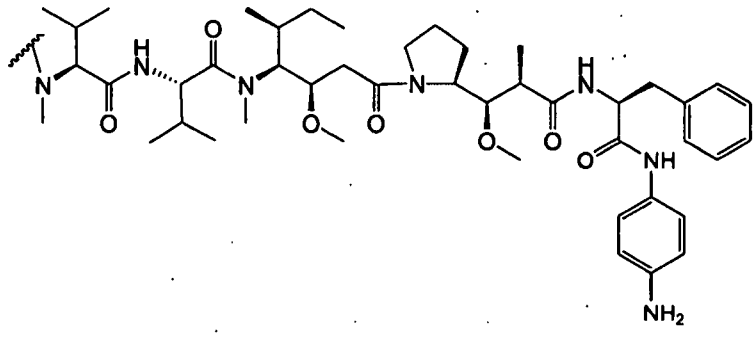


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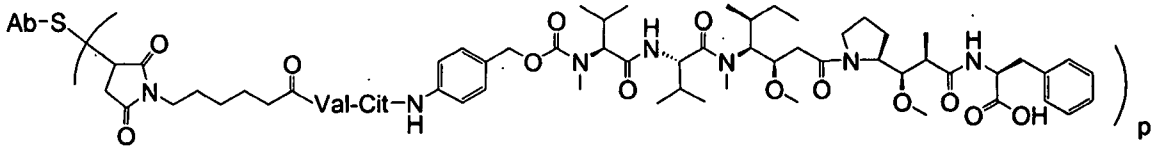
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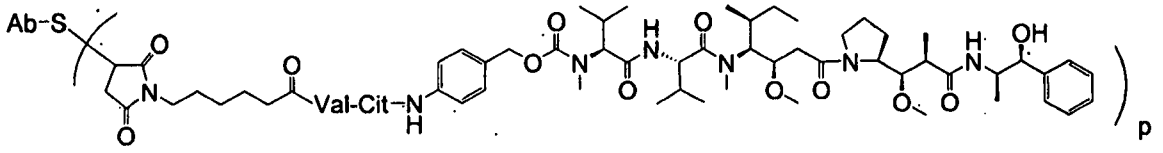
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In one aspect, hydrophilic groups including but not limited to, triethylene glycol esters (TEG), as shown above, can be attached to the drug moiety at R^{11}. Without being bound by any particular theory, the hydrophilic groups assist in the internalization and non-agglomeration of the drug moiety.

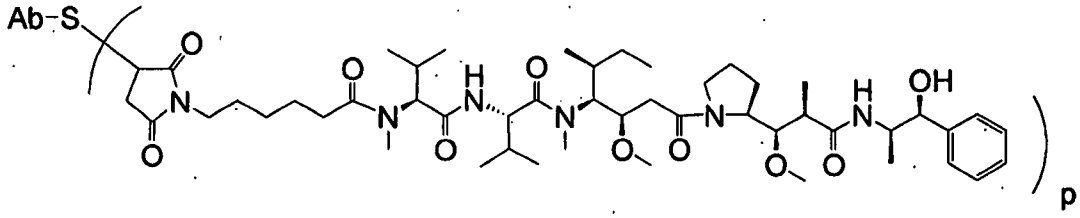
Exemplary embodiments of ADCs of Formula I comprising an auristatin/dolastatin or derivative thereof are described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124, which is expressly incorporated herein by reference. Exemplary embodiments of ADCs of Formula I comprising MMAE or MMAF and various linker components have the following structures and abbreviations (wherein "Ab" is an antibody; p is 1 to about 8, "Val-Cit" is a valine-citrulline dipeptide; and "S" is a sulfur atom:



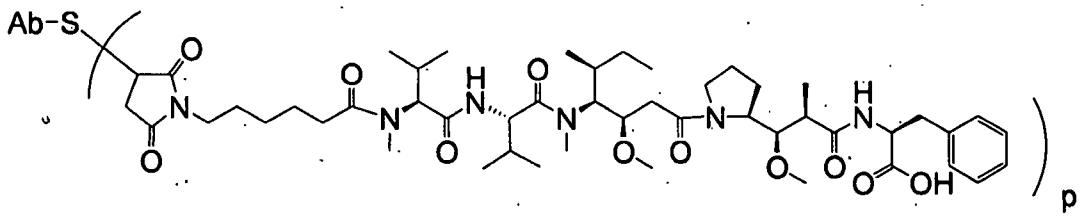
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

Exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Interestingly, immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess

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activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker. See, Doronina et al. (2006) Bioconjugate Chem. 17:114-124. In such instances, drug release is believed to be effected by antibody degradation in the cell. Id.

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. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. Auristatin/dolastatin drug moieties may be prepared according to the methods of: US 2005-0238649 A1; US Pat. No.5635483; US Pat. No.5780588; 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.

In particular, auristatin/dolastatin drug moieties of formula D_F, such as MMAF and derivatives thereof, may be prepared using methods described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124. Auristatin/dolastatin drug moieties of formula D_E, such as MMAE and derivatives thereof, may be prepared using methods described in Doronina et al. (2003) Nat. Biotech. 21:778-784. Drug-linker moieties MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE may be conveniently synthesized by routine methods, e.g., as described in Doronina et al. (2003) Nat. Biotech. 21:778-784, and Patent Application Publication No. US 2005/0238649 A1, and then conjugated to an antibody of interest.

Drug Loading

Drug loading is represented by p and is the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I 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, ELISA assay, and HPLC. 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 reverse phase HPLC or electrophoresis.

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

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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).

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.

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)).

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 (see, e.g., Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

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Certain Methods of Preparing Immunconjugates

An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in US 20050238649 A1, which is expressly incorporated herein by reference.

Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic subsituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug

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(Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

Nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following cross-linker reagents: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA; SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A; see pages 467-498, 2003-2004 Applications Handbook and Catalog.

Immunoconjugates comprising an antibody and a cytotoxic agent may also be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

Alternatively, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a radionucleotide).

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Preparation of cysteine engineered anti-STEAP-1 antibodies

The design, selection, and preparation methods of the invention further enable cysteine engineered anti-STEAP-1 antibodies which are reactive with electrophilic functionality. These methods further enable antibody conjugate compounds such as antibody-drug conjugate (ADC) compounds with drug molecules at designated, designed, selective sites. Reactive cysteine residues on an antibody surface allow specifically conjugating a drug moiety through a thiol reactive group such as maleimide or haloacetyl. The nucleophilic reactivity of the thiol functionality of a Cys residue to a maleimide group is about 1000 times higher compared to any other amino acid functionality in a protein, such as amino group of lysine residues or the N-terminal amino group. Thiol specific functionality in iodoacetyl and maleimide reagents may react with amine groups, but higher pH (>9.0) and longer reaction times are required (Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London). The amount of free thiol in a protein may be estimated by the standard Ellman's assay. Immunoglobulin M is an example of a disulfide-linked pentamer, while immunoglobulin G is an example of a protein with internal disulfide bridges bonding the subunits together. In proteins such as this, reduction of the disulfide bonds with a reagent such as dithiothreitol (DTT) or selenol (Singh et al (2002) Anal. Biochem. 304:147-156) is required to generate the reactive free thiol. This approach may result in loss of antibody tertiary structure and antigen binding specificity.

The Pheselector (Phage ELISA for Selection of Reactive Thiols) Assay allows for detection of reactive cysteine groups in antibodies in an ELISA phage format thereby assisting in the design of cysteine engineered antibodies (WO 2006/034488). The cysteine engineered antibody is coated on well surfaces, followed by incubation with phage particles, addition of HRP labeled secondary antibody, and absorbance detection. Mutant proteins displayed on phage may be screened in a rapid, robust, and high-throughput manner. Libraries of cysteine engineered antibodies can be produced and subjected to binding selection using the same approach to identify appropriately reactive sites of free Cys incorporation from random protein-phage libraries of antibodies or other proteins. This technique includes reacting cysteine mutant proteins displayed on phage with an affinity reagent or reporter group which is also thiol-reactive.

The PHESELECTOR assay allows screening of reactive thiol groups in antibodies. Identification of the A121C variant by this method is exemplary. The entire Fab molecule may be effectively searched to identify more ThioFab variants with reactive thiol groups. A parameter, fractional surface accessibility, was employed to identify and quantitate the accessibility of solvent to the amino acid residues in a polypeptide. The surface accessibility can be expressed as the surface area ($Å^2$) that can be contacted by a solvent molecule, e.g. water. The occupied space of water is approximated as a 1.4 Å radius sphere. Software is freely available or licensable (Secretary to CCP4, Daresbury Laboratory, Warrington, WA4 4AD, United Kingdom, Fax: (+44) 1925 603825, or by internet: www.ccp4.ac.uk/dist/html/INDEX.html)

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as the CCP4 Suite of crystallography programs which employ algorithms to calculate the surface accessibility of each amino acid of a protein with known x-ray crystallography derived coordinates ("The CCP4 Suite: Programs for Protein Crystallography" (1994) Acta. Cryst. D50:760-763). Two exemplary software modules that perform surface accessibility calculations are "AREAIMOL" and "SURFACE", based on the algorithms of B.Lee and F.M.Richards (1971) J.Mol.Biol. 55:379-400. AREAIMOL defines the solvent accessible surface of a protein as the locus of the centre of a probe sphere (representing a solvent molecule) as it rolls over the Van der Waals surface of the protein. AREAIMOL calculates the solvent accessible surface area by generating surface points on an extended sphere about each atom (at a distance from the atom centre equal to the sum of the atom and probe radii), and eliminating those that lie within equivalent spheres associated with neighboring atoms. AREAIMOL finds the solvent accessible area of atoms in a PDB coordinate file, and summarizes the accessible area by residue, by chain and for the whole molecule. Accessible areas (or area differences) for individual atoms can be written to a pseudo-PDB output file. AREAIMOL assumes a single radius for each element, and only recognizes a limited number of different elements.

AREAIMOL and SURFACE report absolute accessibilities, i.e. the number of square Angstroms (Å). Fractional surface accessibility is calculated by reference to a standard state relevant for an amino acid within a polypeptide. The reference state is tripeptide Gly-X-Gly, where X is the amino acid of interest, and the reference state should be an 'extended' conformation, i.e. like those in beta-strands. The extended conformation maximizes the accessibility of X. A calculated accessible area is divided by the accessible area in a Gly-X-Gly tripeptide reference state and reports the quotient, which is the fractional accessibility. Percent accessibility is fractional accessibility multiplied by 100. Another exemplary algorithm for calculating surface accessibility is based on the SOLV module of the program xsae (Broger, C., F. Hoffman-LaRoche, Basel) which calculates fractional accessibility of an amino acid residue to a water sphere based on the X-ray coordinates of the polypeptide. The fractional surface accessibility for every amino acid in an antibody may be calculated using available crystal structure information (Eigenbrot et al. (1993) J Mol Biol. 229:969-995).

DNA encoding the cysteine engineered antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or other mammalian host cells, such as myeloma cells (US 5807715; US 2005/0048572; US 2004/0229310) that do not otherwise produce the antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.

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After design and selection, cysteine engineered antibodies, e.g. ThioFabs, with the engineered, highly reactive unpaired Cys residues, may be produced by: (i) expression in a bacterial, e.g. *E. coli*, system (Skerra et al (1993) Curr. Opinion in Immunol. 5:256-262; Plückthun (1992) Immunol. Revs. 130:151-188) or a mammalian cell culture system (WO 01/00245), e.g. Chinese Hamster Ovary cells (CHO); and (ii) purification using common protein purification techniques (Lowman et al (1991) J. Biol. Chem. 266(17):10982-10988).

The engineered Cys thiol groups react with electrophilic linker reagents and drug-linker intermediates to form cysteine engineered antibody drug conjugates and other labelled cysteine engineered antibodies. Cys residues of cysteine engineered antibodies, and present in the parent antibodies, which are paired and form interchain and intrachain disulfide bonds do not have any reactive thiol groups (unless treated with a reducing agent) and do not react with electrophilic linker reagents or drug-linker intermediates. The newly engineered Cys residue, can remain unpaired, and able to react with, i.e. conjugate to, an electrophilic linker reagent or drug-linker intermediate, such as a drug-maleimide. Exemplary drug-linker intermediates include: MC-MMAE, MC-MMAF, MC-vc-PAB-MMAE, and MC-vc-PAB-MMAF. The structure positions of the engineered Cys residues of the heavy and light chains are numbered according to a sequential numbering system. This sequential numbering system is correlated to the Kabat numbering system (Kabat et al., (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) starting at the N-terminus, differs from the Kabat numbering scheme (bottom row) by insertions noted by a,b,c. Using the Kabat numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. The cysteine engineered heavy chain variant sites are identified by the sequential numbering and Kabat numbering schemes.

In one embodiment, the cysteine engineered anti-STEAP-1 antibody is prepared by a process comprising:

(a) replacing one or more amino acid residues of a parent anti-STEAP-1 antibody by cysteine; and

(b) determining the thiol reactivity of the cysteine engineered anti-STEAP-1 antibody by reacting the cysteine engineered antibody with a thiol-reactive reagent.

The cysteine engineered antibody may be more reactive than the parent antibody with the thiol-reactive reagent.

The free cysteine amino acid residues may be located in the heavy or light chains, or in the constant or variable domains. Antibody fragments, e.g. Fab, may also be engineered with one or more cysteine amino acids replacing amino acids of the antibody fragment, to form cysteine engineered antibody fragments.

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Another embodiment of the invention provides a method of preparing (making) a cysteine engineered anti-STEAP-1 antibody, comprising:

(a) introducing one or more cysteine amino acids into a parent anti-STEAP-1 antibody in order to generate the cysteine engineered anti-STEAP-1 antibody; and

(b) determining the thiol reactivity of the cysteine engineered antibody with a thiol-reactive reagent;

wherein the cysteine engineered antibody is more reactive than the parent antibody with the thiol-reactive reagent.

Step (a) of the method of preparing a cysteine engineered antibody may comprise:

(i) mutagenizing a nucleic acid sequence encoding the cysteine engineered antibody;

(ii) expressing the cysteine engineered antibody; and

(iii) isolating and purifying the cysteine engineered antibody.

Step (b) of the method of preparing a cysteine engineered antibody may comprise expressing the cysteine engineered antibody on a viral particle selected from a phage or a phagemid particle.

Step (b) of the method of preparing a cysteine engineered antibody may also comprise:

(i) reacting the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody; and

(ii) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media.

Another embodiment of the invention is a method of screening cysteine engineered antibodies with highly reactive, unpaired cysteine amino acids for thiol reactivity comprising:

(a) introducing one or more cysteine amino acids into a parent antibody in order to generate a cysteine engineered antibody;

(b) reacting the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody; and

(c) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media; and

(d) determining the thiol reactivity of the cysteine engineered antibody with the thiol-reactive reagent.

Step (a) of the method of screening cysteine engineered antibodies may comprise:

(i) mutagenizing a nucleic acid sequence encoding the cysteine engineered antibody;

(ii) expressing the cysteine engineered antibody; and

(iii) isolating and purifying the cysteine engineered antibody.

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Step (b) of the method of screening cysteine engineered antibodies may comprise expressing the cysteine engineered antibody on a viral particle selected from a phage or a phagemid particle.

Step (b) of the method of screening cysteine engineered antibodies may also comprise:

(i) reacting the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody; and

(ii) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media.

Labelled cysteine engineered anti-STEAP-1 antibodies

Cysteine engineered anti-STEAP-1 antibodies may be site-specifically and efficiently coupled with a thiol-reactive reagent. The thiol-reactive reagent may be a multifunctional linker reagent, a capture, i.e. affinity, label reagent (e.g. a biotin-linker reagent), a detection label (e.g. a fluorophore reagent), a solid phase immobilization reagent (e.g. SEPHAROSE™, polystyrene, or glass), or a drug-linker intermediate. One example of a thiol-reactive reagent is N-ethyl maleimide (NEM). In an exemplary embodiment, reaction of a ThioFab with a biotin-linker reagent provides a biotinylated ThioFab by which the presence and reactivity of the engineered cysteine residue may be detected and measured. Reaction of a ThioFab with a multifunctional linker reagent provides a ThioFab with a functionalized linker which may be further reacted with a drug moiety reagent or other label. Reaction of a ThioFab with a drug-linker intermediate provides a ThioFab drug conjugate.

The exemplary methods described here may be applied generally to the identification and production of antibodies, and more generally, to other proteins through application of the design and screening steps described herein.

Such an approach may be applied to the conjugation of other thiol-reactive reagents in which the reactive group is, for example, a maleimide, an iodoacetamide, a pyridyl disulfide, or other thiol-reactive conjugation partner (Haugland, 2003, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc.; Brinkley, 1992, Bioconjugate Chem. 3:2; Garman, 1997, Non-Radioactive Labelling: A Practical Approach, Academic Press, London; Means (1990) Bioconjugate Chem. 1:2; Hermanson, G. in Bioconjugate Techniques (1996) Academic Press, San Diego, pp. 40-55, 643-671). The thiol-reactive reagent may be a drug moiety, a fluorophore such as a fluorescent dye like fluorescein or rhodamine, a chelating agent for an imaging or radiotherapeutic metal, a peptidyl or non-peptidyl label or detection tag, or a clearance-modifying agent such as various isomers of polyethylene glycol, a peptide that binds to a third component, or another carbohydrate or lipophilic agent.

Uses of cysteine engineered anti-STEAP-1 antibodies

Cysteine engineered anti-STEAP-1 antibodies, and conjugates thereof may find use as therapeutic and/or diagnostic agents. The present invention further provides methods of preventing, managing,

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treating or ameliorating one or more symptoms associated with a STEAP-1 related disorder. In particular, the present invention provides methods of preventing, managing, treating, or ameliorating one or more symptoms associated with a cell proliferative disorder, such as cancer, e.g., prostate cancer, lung cancer. colon cancer, bladder cancer, ovarian cancer, and Ewing's sarcoma. The present invention still further provides methods for diagnosing a STEAP-1 related disorder or predisposition to developing such a disorder, as well as methods for identifying antibodies, and antigen-binding fragments of antibodies, that preferentially bind cell-associated STEAP-1 polypeptides.

Another embodiment of the present invention is directed to the use of a cysteine engineered anti-STEAP-1 antibody for the preparation of a medicament useful in the treatment of a condition which is responsive to a STEAP-1 related disorder.

Preparation of cysteine engineered anti-STEAP-1 antibody-drug conjugates

The ADC of Formula **I** may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a cysteine group of a cysteine engineered antibody with a linker reagent, to form antibody-linker intermediate Ab-L, via a covalent bond, followed by reaction with an activated drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a linker reagent, to form drug-linker intermediate D-L, via a covalent bond, followed by reaction with a cysteine group of a cysteine engineered antibody. Conjugation methods (1) and (2) may be employed with a variety of cysteine engineered antibodies, drug moieties, and linkers to prepare the antibody-drug conjugates of Formula **I**.

Antibody cysteine thiol groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker reagents and drug-linker intermediates including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides, such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups; and (iv) disulfides, including pyridyl disulfides, via sulfide exchange. Nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents.

Cysteine engineered antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (Cleland's reagent, dithiothreitol) or TCEP (tris(2-carboxyethyl)phosphine hydrochloride; Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA), followed by reoxidation to reform interchain and intrachain disulfide bonds (Example x). For example, full length, cysteine engineered monoclonal antibodies (ThioMabs) expressed in CHO cells are reduced with about a 50 fold excess of TCEP for 3 hrs at 37 °C to reduce disulfide bonds in cysteine adducts which may form between the newly introduced cysteine residues and the cysteine present in the

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culture media. The reduced ThioMab is diluted and loaded onto HiTrap S column in 10 mM sodium acetate, pH 5, and eluted with PBS containing 0.3M sodium chloride. Disulfide bonds were reestablished between cysteine residues present in the parent Mab with dilute (200 nM) aqueous copper sulfate ($CuSO_4$) at room temperature, overnight. Alternatively, dehydroascorbic acid (DHAA) is an effective oxidant to reestablish the intrachain disulfide groups of the cysteine engineered antibody after reductive cleavage of the cysteine adducts. Other oxidants, i.e. oxidizing agents, and oxidizing conditions, which are known in the art may be used. Ambient air oxidation is also effective. This mild, partial reoxidation step forms intrachain disulfides efficiently with high fidelity and preserves the thiol groups of the newly introduced cysteine residues. An approximate 10 fold excess of drug-linker intermediate, e.g. MC-vc-PAB-MMAE, was added, mixed, and let stand for about an hour at room temperature to effect conjugation and form the SPECIFIC CLONE anti-____ antibody-drug conjugate. The conjugation mixture was gel filtered and loaded and eluted through a HiTrap S column to remove excess drug-linker intermediate and other impurities.

Figure 16 shows the general process to prepare a cysteine engineered antibody expressed from cell culture for conjugation. When the cell culture media contains cysteine, disulfide adducts can form between the newly introduced cysteine amino acid and cysteine from media. These cysteine adducts, depicted as a circle in the exemplary ThioMab (left) in Figure 12, must be reduced to generate cysteine engineered antibodies reactive for conjugation. Cysteine adducts, presumably along with various interchain disulfide bonds, are reductively cleaved to give a reduced form of the antibody with reducing agents such as TCEP. The interchain disulfide bonds between paired cysteine residues are reformed under partial oxidation conditions with copper sulfate, DHAA, or exposure to ambient oxygen. The newly introduced, engineered, and unpaired cysteine residues remain available for reaction with linker reagents or drug-linker intermediates to form the antibody conjugates of the invention. The ThioMabs expressed in mammalian cell lines result in externally conjugated Cys adduct to an engineered Cys through –S-S- bond formation. Hence the purified ThioMabs are treated with the reduction and reoxidation procedures as described in Example x to produce reactive ThioMabs. These ThioMabs are used to conjugate with maleimide containing cytotoxic drugs, fluorophores, and other labels.

Figure 15 shows embodiments of cysteine engineered anti-STEAP-1 antibody drug conjugates (ADC) where an auristatin drug moiety is attached to an engineered cysteine group in: the light chain (LC-ADC); the heavy chain (HC-ADC); and the Fc region (Fc-ADC).

Pharmaceutical Formulations

Administration of Antibody-Drug Conjugates

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The antibody-drug conjugates (ADC) of the invention may be administered by any route appropriate to the condition to be treated. The ADC will typically be administered parenterally, i.e. infusion, subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural.

For treating cancers of, for example, prostate, lung and/or colon, in one embodiment, the antibody-drug conjugate is administered via intravenous infusion. The dosage administered via infusion is in the range of about 1 $\mu g/m^2$ to about 10,000 $\mu g/m^2$ per dose, generally one dose per week for a total of one, two, three or four doses. Alternatively, the dosage range is of about 1 $\mu g/m^2$ to about 1000 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 800 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 600 $\mu g/m^2$, about 1 $\mu g/m^2$ to about 400 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 500 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 300 $\mu g/m^2$, about 10 $\mu g/m^2$ to about 200 $\mu g/m^2$, and about 1 $\mu g/m^2$ to about 200 $\mu g/m^2$. The dose may be administered once per day, once per week, multiple times per week, but less than once per day, multiple times per month but less than once per day, multiple times per month but less than once per week, once per month or intermittently to relieve or alleviate symptoms of the disease. Administration may continue at any of the disclosed intervals until remission of the tumor or symptoms of the lymphoma, leukemia being treated. Administration may continue after remission or relief of symptoms is achieved where such remission or relief is prolonged by such continued administration.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding embodiments, which antibody is not conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

The invention also provides a method of treating a prostate, lung, and/or colon cancer, and/or a metastasis of such cancer, comprising administering to a patient suffering from a prostate, lung or colon cancer, a therapeutically effective amount of a humanized 120v.24 antibody of any one of the preceding embodiments, which antibody is conjugated to a cytotoxic molecule or a detectable molecule. The antibody will typically be administered in a dosage range of about 1 $\mu g/m^2$ to about 1000 mg/m^2.

In one aspect, the invention further provides pharmaceutical formulations comprising at least one anti-STEAP-1 antibody of the invention and/or at least one immunoconjugate thereof and/or at least one anti-STEAP-1 antibody-drug conjugate of the invention. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an anti-STEAP-1 antibody-drug conjugate and/or an immunoconjugate thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an anti-STEAP-1 antibody and/or an immunoconjugate thereof, and optionally, 2) at least one additional therapeutic agent.

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Pharmaceutical formulations comprising an antibody or immunoconjugate of the invention or the antibody-drug conjugate of the invention are prepared for storage by mixing the antibody or antibody-drug conjugate having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)) in the form of aqueous solutions or lyophilized or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, histidine 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). Pharmaceutical formulations to be used for in vivo administration are generally sterile. This is readily accomplished by filtration through sterile filtration membranes.

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

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

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immunoconjugates remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, 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.

Antibody-Drug Conjugate Treatments

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant tumors; leukemia and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders. Still others include prostate, lung and colon cancers.

The ADC compounds which are identified in the animal models and cell-based assays can be further tested in tumor-bearing higher primates and human clinical trials. Human clinical trials can be designed to test the efficacy of the anti-STEAP-1 monoclonal antibody or immunoconjugate of the invention in patients experiencing a prostate, lung or colon cell proliferative disorder including without limitation prostate, lung and colon cancers and metastases of such cancers. The clinical trial may be designed to evaluate the efficacy of an ADC in combinations with known therapeutic regimens, such as radiation and/or chemotherapy involving known chemotherapeutic and/or cytotoxic agents.

The cancer may comprise STEAP-1-expressing cells, such that an ADC of the present invention is able to bind to the cancer cells. To determine STEAP-1 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, STEAP-1 overexpression may be analyzed by IHC. Parrafin-embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a STEAP-1 protein staining intensity criteria with respect to the degree of staining and in what proportion of tumor cells examined.

For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by

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continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight.

For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of an anti-STEAP-1 antibody. Other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Combination Therapy

An antibody-drug conjugate (ADC) of the invention may be combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with at least one additional compound having anti-cancer properties. The at lelast one additional compound of the pharmaceutical combination formulation or dosing regimen preferably has complementary activities to the ADC of the combination such that they do not adversely affect each other.

The at least one additional compound may be a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. A pharmaceutical composition containing an ADC of the invention may also have a therapeutically effective amount of a chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, or a DNA binder.

In one aspect, the first compound is an anti-STEAP-1 ADC of the invention and the at least one additional compound is a therapeutic antibody other than an anti-STEAP-1 (naked antibody or an ADC). In one embodiment, the at least one additional compound is an anti-PSCA antibody. In one embodiment the at least one additional compound is an anti-HER2 antibody, trastuzumab (e.g., Herceptin®, Genentech, Inc., South San Francisco, CA). In one embodiment the at least one additional compound is an anti-HER2 antibody, pertuzumab (Omnitarg™, Genentech, Inc., South San Francisco, CA, see US6949245). In one embodiment, the at least one additional compound is an anti-VEGF antibody (e.g, Avastin®, Genentech, Inc.). In each case, the at least one compound is either a naked antibody or an ADC). In an embodiment, the at least one additional compound is an antibody (either a naked antibody or an ADC), and the additional antibody is a second, third, fourth, fifth, sixth antibody or more, such that a combination of such second, third, fourth, fifth, sixth, or more antibodies (either naked or as an ADC) is efficacious in treating a cell proliferative disease in a tissue expressing STEAP-1.

Other therapeutic regimens may be combined with the administration of an anticancer agent identified in accordance with this invention, including without limitation radiation therapy and/or bone marrow and peripheral blood transplants, and/or a cytotoxic agent, a chemotherapeutic agent, or a growth

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inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA, anti-HER2 (e.g., Herceptin®, Omnitarg™) or anti-VEGF (e.g., Avastin®). The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one embodiment, treatment with an ADC involves the combined administration of an anticancer agent identified herein, and one or more chemotherapeutic agents or growth inhibitory agents, including coadministration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include taxanes (such as paclitaxel and docetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in "Chemotherapy Service", (1992) Ed., M.C. Perry, Williams & Wilkins, Baltimore, Md.

Suitable dosages for any of the above coadministered agents are those presently used and may be lowered due to the combined action (synergy) of the newly identified agent and other chemotherapeutic agents or treatments.

The combination therapy may provide "synergy" and prove "synergistic", i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

Metabolites of the Antibody-Drug Conjugates

Also falling within the scope of this invention are the in vivo metabolic products of the ADC compounds described herein, to the extent such products are novel and unobvious over the prior art. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification, enzymatic cleavage, and the like, of the administered compound. Accordingly, the invention includes

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novel and unobvious compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof.

Metabolite products typically are identified by preparing a radiolabelled (e.g. 14C or 3H) ADC, administering it parenterally in a detectable dose (e.g. greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g. by MS, LC/MS or NMR analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found in vivo, are useful in diagnostic assays for therapeutic dosing of the ADC compounds of the invention.

Further Methods of Using Anti-STEAP-1 Antibodies and Immunoconjugates

Diagnostic methods and methods of detection

In one aspect, anti-STEAP-1 antibodies and immunoconjugates of the invention are useful for detecting the presence of STEAP-1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue. In certain embodiments, such tissues include normal and/or cancerous tissues that express STEAP-1 at higher levels relative to other tissues, for example, prostate, lung and colon.

In one aspect, the invention provides a method of detecting the presence of STEAP-1 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1.

In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of STEAP-1. In certain embodiments, the method comprises contacting a test cell with an anti-STEAP-1 antibody; determining the level of expression (either quantitatively or qualitatively) of STEAP-1 by the test cell by detecting binding of the anti-STEAP-1 antibody to STEAP-1; and comparing the level of expression of STEAP-1 by the test cell with the level of expression of STEAP-1 by a control cell (e.g., a normal cell of the same tissue origin as the test cell or a cell that expresses STEAP-1 at levels comparable to such a normal cell), wherein a higher level of expression of STEAP-1 by the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of STEAP-1. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated

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with increased expression of STEAP-1. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor.

Exemplary cell proliferative disorders that may be diagnosed using an antibody of the invention include a prostate, lung and colon cancers or metastases of such cancers.

In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface. In certain embodiments, the method comprises contacting a cell with an anti-STEAP-1 antibody under conditions permissive for binding of the anti-STEAP-1 antibody to STEAP-1, and detecting whether a complex is formed between the anti-STEAP-1 antibody and STEAP-1 on the cell surface. An exemplary assay for detecting binding of an anti-STEAP-1 antibody to STEAP-1 expressed STEAP-1 on the surface of a cell is a "FACS" assay.

Certain other methods can be used to detect binding of anti-STEAP-1 antibodies to STEAP-1. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

In certain embodiments, anti-STEAP-1 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

In certain embodiments, anti-STEAP-1 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-STEAP-1 antibody from any STEAP-1 that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-STEAP-1 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al.., U.S. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing

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the anti-STEAP-1 antibody after formation of a complex between the anti-STEAP-1 antibody and STEAP-1, e.g., by immunoprecipitation.

Any of the above embodiments of diagnosis or detection may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-STEAP-1 antibody.

Therapeutic methods

An antibody or immunoconjugate of the invention may be used in, for example, in vitro, ex vivo, and in vivo therapeutic methods. In one aspect, the invention provides methods for inhibiting cell growth or proliferation, either in vivo or in vitro, the method comprising exposing a cell to an anti-STEAP-1 antibody or immunoconjugate thereof under conditions permissive for binding of the immunoconjugate to STEAP-1. "Inhibiting cell growth or proliferation" means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death. In certain embodiments, the cell is a tumor cell. In certain embodiments, the cell is a prostate, lung, colon, bladder, or ovary cell, or Ewing's sarcoma cell. In certain embodiments, the cell is a xenograft, e.g., as exemplified herein.

In one aspect, an antibody or immunoconjugate of the invention is used to treat or prevent a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder. In certain embodiments, the cell proliferative disorder is associated with increased expression and/or activity of STEAP-1. For example, in certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is associated with increased expression of STEAP-1 on the surface of a prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell. In certain embodiments, the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell proliferative disorder is a tumor or a cancer or metastasis of such cancer.

In one aspect, the invention provides methods for treating a prostate, lung or colon cell proliferative disorder comprising administering to an individual an effective amount of an anti-STEAP-1 antibody or immunoconjugate thereof. In certain embodiments, a method for treating a prostate, lung or colon cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising an anti-STEAP-1 antibody or anti-STEAP-1 immunoconjugate and, optionally, at least one additional therapeutic agent, such as those provided herein.

In one aspect, at least some of the antibodies or immunoconjugates of the invention can bind STEAP-1 from species other than human. Accordingly, antibodies or immunoconjugates of the invention can be used to bind STEAP-1, e.g., in a cell culture containing STEAP-1, in humans, or in other mammals having a STEAP-1 with which an antibody or immunoconjugate of the invention cross-reacts (e.g. chimpanzee, baboon, marmoset, cynomolgus and rhesus monkeys, dog, pig, rat, or mouse). In one embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used for targeting STEAP-1 on

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prostate, lung or colono cells by contacting the antibody or immunoconjugate with STEAP-1 to form an antibody or immunoconjugate-antigen complex such that a conjugated cytotoxin of the immunoconjugate accesses the interior of the cell. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is human STEAP-1. In one embodiment, the STEAP-1 to which the anti-STEAP-1 antibody binds is cynomolgus monkey STEAP-1. In one embodiment, the humanized anti-STEAP-1 antibody binds to human and/or cynomolgus monkey STEAP-1.

In one embodiment, an anti-STEAP-1 antibody or immunoconjugate can be used in a method for binding STEAP-1 in an individual suffering from a disorder associated with increased STEAP-1 expression and/or activity, the method comprising administering to the individual the antibody or immunoconjugate such that STEAP-1 in the individual is bound. In one embodiment, the bound antibody or immunoconjugate is internalized into the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell expressing STEAP-1. In one embodiment, the STEAP-1 is human STEAP-1, and the individual is a human individual. Alternatively, the individual can be a mammal expressing STEAP-1 to which an anti-STEAP-1 antibody binds. Still further the individual can be a mammal into which STEAP-1 has been introduced (e.g., by administration of STEAP-1 or by expression of a transgene encoding STEAP-1).

An anti-STEAP-1 antibody or immunoconjugate can be administered to a human for therapeutic purposes. Moreover, an anti-STEAP-1 antibody or immunoconjugate can be administered to a non-human mammal expressing STEAP-1 with which the antibody cross-reacts (e.g., a primate, dog, pig, rat, or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies or immunoconjugates of the invention (e.g., testing of dosages and time courses of administration).

Antibodies or immunoconjugates of the invention can be used either alone or in combination with other compositions in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent and/or adjuvant. In certain embodiments, an additional therapeutic agent is a cytotoxic agent, a chemotherapeutic agent, or a growth inhibitory agent. In one of such embodiments, a chemotherapeutic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubincin, vincristine (Oncovin™), prednisolone, CHOP, CVP, or COP, or immunotherapeutics such as anti-PSCA (see, for example, US6824780), anti-VEGF (e.g., Avastin®, Genentech, Inc.), anti-HER2 (e.g., Herceptin®, Omnitarg™ Genentech, Inc.), or anti-HER2 in combination with Taxol® (see, for example, BioWorld Today, November 17, 1999, page 1), wherein the combination therapy is useful in the treatment of cell proliferative disorders, cancers, and/or metastases of cancers of prostate, lung and/or colon.

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Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

An antibody or immunoconjugate of the invention (and any additional therapeutic agent or adjuvant) can be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antibody or immunoconjugate is suitably administered by pulse infusion, particularly with declining doses of the antibody or immunoconjugate. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

Antibodies or immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or immunoconjugate need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or immunoconjugate present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody or immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents, such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of antibody or immunoconjugate, the severity and course of the disease, whether the antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the discretion of the attending physician. The antibody or immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 100 mg/kg (e.g. 0.1mg/kg-20mg/kg) of antibody or immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion.

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One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) of antibody or immunoconjugate may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Assays

Anti-STEAP-1 antibodies and immunoconjugates of the invention may be characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Activity assays

In one aspect, assays are provided for identifying anti-STEAP-1 antibodies or immunoconjugates thereof having biological activity. Biological activity may include, e.g., the ability to inhibit cell growth or proliferation (e.g., "cell killing" activity), or the ability to induce cell death, including programmed cell death (apoptosis). Antibodies or immunoconjugates having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vitro. Assays for inhibition of cell growth or proliferation are well known in the art. Certain assays for cell proliferation, exemplified by the "cell killing" assays described herein, measure cell viability. One such assay is the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. See Crouch et al (1993) J. Immunol. Meth. 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). See Cree et al (1995) AntiCancer Drugs 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be

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recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

Another assay for cell proliferation is the "MTT" assay, a colorimetric assay that measures the oxidation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan by mitochondrial reductase. Like the CellTiter-Glo™ assay, this assay indicates the number of metabolically active cells present in a cell culture. See, e.g., Mosmann (1983) J. Immunol. Meth. 65:55-63, and Zhang et al. (2005) Cancer Res. 65:3877-3882.

In one aspect, an anti-STEAP-1 antibody is tested for its ability to induce cell death in vitro. Assays for induction of cell death are well known in the art. In some embodiments, such assays measure, e.g., loss of membrane integrity as indicated by uptake of propidium iodide (PI), trypan blue (see Moore et al. (1995) Cytotechnology, 17:1-11), or 7AAD. In an exemplary PI uptake assay, cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. Thus, the assay is performed in the absence of complement and immune effector cells. Cells are seeded at a density of 3×10^6 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is removed and replaced with fresh medium alone or medium containing various concentrations of the antibody or immunoconjugate. The cells are incubated for a 3-day time period. Following treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4 °C, the pellet resuspended in 3 ml cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12 x 75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 µg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Antibodies or immunoconjugates which induce statistically significant levels of cell death as determined by PI uptake are thus identified.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate is tested for its ability to induce apoptosis (programmed cell death) in vitro. An exemplary assay for antibodies or immunconjugates that induce apoptosis is an annexin binding assay. In an exemplary annexin binding assay, cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is removed and replaced with fresh medium alone or medium containing 0.001 to 10 µg/ml of the antibody or immunoconjugate. Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca^{2+} binding buffer, and aliquoted into tubes as discussed in the preceding paragraph. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 µg/ml). Samples are analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (BD Biosciences). Antibodies or immunoconjugates that induce statistically significant levels of annexin binding relative to control are thus identified. Another exemplary assay for antibodies or

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immunconjugates that induce apoptosis is a histone DNA ELISA colorimetric assay for detecting internucleosomal degradation of genomic DNA. Such an assay can be performed using, e.g., the Cell Death Detection ELISA kit (Roche, Palo Alto, CA).

Cells for use in any of the above in vitro assays include cells or cell lines that naturally express STEAP-1 or that have been engineered to express STEAP-1. Such cells include tumor cells that overexpress STEAP-1 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express STEAP-1 and cell lines that do not normally express STEAP-1 but have been transfected with nucleic acid encoding STEAP-1.

In one aspect, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-STEAP-1 antibody or immunoconjugate thereof is tested for its ability to inhibit tumor growth in vivo. In vivo model systems, such as xenograft models, can be used for such testing. In an exemplary xenograft system, human tumor cells are introduced into a suitably immunocompromised non-human animal, e.g., a SCID mouse. An antibody or immunoconjugate of the invention is administered to the animal. The ability of the antibody or immunoconjugate to inhibit or decrease tumor growth is measured. In certain embodiments of the above xenograft system, the human tumor cells are tumor cells from a human patient. Such cells useful for preparing xenograft models include human prostate, lung, or colon tumor cell lines, which include without limitation PC3 cells expressing exogenous STEAP-1, and cells naturally expressing STEAP-1 which include, without limitation, LnCAP cells (Southern Research Institute, Birmingham, AL), LuCAP 77 cells, and LuCAP35V cells (University of Washington, Seattle, WA). In certain embodiments, the human tumor cells are introduced into a suitably immunocompromised non-human animal by subcutaneous injection or by transplantation into a suitable site, such as a mammary fat pad.

Binding assays and other assays

In one aspect, an anti-STEAP-1 antibody is tested for its antigen binding activity. For example, in certain embodiments, an anti-STEAP-1 antibody is tested for its ability to bind to exogenous or endogenous STEAP-1 expressed on the surface of a cell. A FACS assay may be used for such testing.

In one aspect, competition assays may be used to identify a monoclonal antibody that competes with 120 graft or humanized variants thereof, including without limitation, 120v.24 antibody for binding to STEAP-1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear epitope peptide or a conformational epitope formed by expression of STEAP1 on a cell surface) that is bound by 120 graft antibody, or humanized 120 graft antibody, including variant 120v.24 humanized anti-STEAP-1 antibody. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Detailed exemplary methods for mapping an epitope to which an

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antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). Two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more.

In an exemplary competition assay, immobilized STEAP-1 is incubated in a solution comprising a first labeled antibody that binds to STEAP-1 (e.g., murine 120.545 antibody, 120 graft antibody, or humanized 120v.24 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to STEAP-1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized STEAP-1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to STEAP-1, excess unbound antibody is removed, and the amount of label associated with immobilized STEAP-1 is measured. If the amount of label associated with immobilized STEAP-1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to STEAP-1. In certain embodiments, immobilized STEAP-1 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing STEAP-1 on its surface.

In one aspect, purified anti-STEAP-1 antibodies can be further characterized by a series of assays including, but not limited to, N-terminal sequencing, amino acid analysis, non-denaturing size exclusion high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography and papain digestion.

In one embodiment, the invention contemplates an altered antibody that possesses some but not all effector functions, which make it a desirable candidate for many applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In certain embodiments, the Fc activities of the antibody are measured to ensure that only the desired properties are maintained. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-92 (1991). An example of an in vitro assay to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998). C1q binding assays may also be carried out to

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confirm that the antibody is unable to bind C1q and hence lacks CDC activity. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed. FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art.

EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1: Preparation of Humanized anti-STEAP-1 antibodies

Nucleic acid molecules encoding amino acid sequence variants of the antibody, antibody fragment, VL domain or VH domain are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody, antibody fragment, VL domain or VH domain. For example, libraries can be created by targeting VL accessible amino acid positions in VH, and optionally in one or more CDRs, for amino acid substitution with variant amino acids using the Kunkel method. See, for e.g., Kunkel et al., Methods Enzymol. (1987), 154:367-382 and the examples herein. Generation of randomized sequences is also described below in the Examples.

The sequence of oligonucleotides includes one or more of the designed codon sets for a particular position in a CDR (HVR) or FR region of a polypeptide of the invention. A codon set is a set of different nucleotide triplet sequences used to encode desired variant amino acids. Codon sets can be represented using symbols to designate particular nucleotides or equimolar mixtures of nucleotides as shown in below according to the IUB code.

IUB CODES

G Guanine
A Adenine
T Thymine
C Cytosine
R (A or G)
Y (C or T)
M (A or C)
K (G or T)
S (C or G)
W (A or T)

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H (A or C or T)

B (C or G or T)

V (A or C or G)

D (A or G or T)

N (A or C or G or T)

For example, in the codon set DVK, D can be nucleotides A or G or T; V can be A or G or C; and K can be G or T. This codon set can present 18 different codons and can encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys.

Oligonucleotide or primer sets can be synthesized using standard methods. A set of oligonucleotides can be synthesized, for example, by solid phase synthesis, containing sequences that represent all possible combinations of nucleotide triplets provided by the codon set and that will encode the desired group of amino acids. Synthesis of oligonucleotides with selected nucleotide "degeneracy" at certain positions is well known in that art. Such sets of nucleotides having certain codon sets can be synthesized using commercial nucleic acid synthesizers (available from, for example, Applied Biosystems, Foster City, CA), or can be obtained commercially (for example, from Life Technologies, Rockville, MD). Therefore, a set of oligonucleotides synthesized having a particular codon set will typically include a plurality of oligonucleotides with different sequences, the differences established by the codon set within the overall sequence. Oligonucleotides, as used according to the invention, have sequences that allow for hybridization to a variable domain nucleic acid template and also can include restriction enzyme sites for cloning purposes.

In one method, nucleic acid sequences encoding variant amino acids can be created by oligonucleotide-mediated mutagenesis. This technique is well known in the art as described by Zoller et al, 1987, Nucleic Acids Res. 10:6487-6504. Briefly, nucleic acid sequences encoding variant amino acids are created by hybridizing an oligonucleotide set encoding the desired codon sets to a DNA template, where the template is the single-stranded form of the plasmid containing a variable region nucleic acid template sequence. After hybridization, DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will contain the codon sets as provided by the oligonucleotide set.

Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation(s). This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al., Proc. Nat'l. Acad. Sci. USA, 75:5765 (1978).

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The DNA template is generated by those vectors that are either derived from bacteriophage M13 vectors (the commercially available M13mp18 and M13mp19 vectors are suitable), or those vectors that contain a single-stranded phage origin of replication as described by Viera et al., Meth. Enzymol., 153:3 (1987). Thus, the DNA that is to be mutated can be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., above.

To alter the native DNA sequence, the oligonucleotide is hybridized to the single stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually T7 DNA polymerase or the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of gene 1, and the other strand (the original template) encodes the native, unaltered sequence of gene 1. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as E. coli JM101. After growing the cells, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabelled with a 32-Phosphate to identify the bacterial colonies that contain the mutated DNA.

The method described immediately above may be modified such that a homoduplex molecule is created wherein both strands of the plasmid contain the mutation(s). The modifications are as follows: The single stranded oligonucleotide is annealed to the single-stranded template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTT), is combined with a modified thiodeoxyribocytosine called dCTP-(aS) (which can be obtained from Amersham). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated bases is generated. In addition, this new strand of DNA will contain dCTP-(aS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell.

As indicated previously the sequence of the oligonucleotide set is of sufficient length to hybridize to the template nucleic acid and may also, but does not necessarily, contain restriction sites. The DNA template can be generated by those vectors that are either derived from bacteriophage M13 vectors or vectors that contain a single-stranded phage origin of replication as described by Viera et al. ((1987) Meth.

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Enzymol., 153:3). Thus, the DNA that is to be mutated must be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., supra.

According to another method, a library can be generated by providing upstream and downstream oligonucleotide sets, each set having a plurality of oligonucleotides with different sequences, the different sequences established by the codon sets provided within the sequence of the oligonucleotides. The upstream and downstream oligonucleotide sets, along with a variable domain template nucleic acid sequence, can be used in a polymerase chain reaction to generate a "library" of PCR products. The PCR products can be referred to as "nucleic acid cassettes", as they can be fused with other related or unrelated nucleic acid sequences, for example, viral coat proteins and dimerization domains, using established molecular biology techniques.

Oligonucleotide sets can be used in a polymerase chain reaction using a variable domain nucleic acid template sequence as the template to create nucleic acid cassettes. The variable domain nucleic acid template sequence can be any portion of the heavy immunoglobulin chains containing the target nucleic acid sequences (ie., nucleic acid sequences encoding amino acids targeted for substitution). The variable region nucleic acid template sequence is a portion of a double stranded DNA molecule having a first nucleic acid strand and complementary second nucleic acid strand. The variable domain nucleic acid template sequence contains at least a portion of a variable domain and has at least one CDR. In some cases, the variable domain nucleic acid template sequence contains more than one CDR. An upstream portion and a downstream portion of the variable domain nucleic acid template sequence can be targeted for hybridization with members of an upstream oligonucleotide set and a downstream oligonucleotide set.

A first oligonucleotide of the upstream primer set can hybridize to the first nucleic acid strand and a second oligonucleotide of the downstream primer set can hybridize to the second nucleic acid strand. The oligonucleotide primers can include one or more codon sets and be designed to hybridize to a portion of the variable region nucleic acid template sequence. Use of these oligonucleotides can introduce two or more codon sets into the PCR product (i.e., the nucleic acid cassette) following PCR. The oligonucleotide primer that hybridizes to regions of the nucleic acid sequence encoding the antibody variable domain includes portions that encode CDR residues that are targeted for amino acid substitution.

The upstream and downstream oligonucleotide sets can also be synthesized to include restriction sites within the oligonucleotide sequence. These restriction sites can facilitate the insertion of the nucleic acid cassettes (i.e., PCR reaction products) into an expression vector having additional antibody sequence. In one embodiment, the restriction sites are designed to facilitate the cloning of the nucleic acid cassettes without introducing extraneous nucleic acid sequences or removing original CDR or framework nucleic acid sequences.

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Nucleic acid cassettes can be cloned into any suitable vector for expression of a portion or the entire light or heavy chain sequence containing the targeted amino acid substitutions generated via the PCR reaction. According to methods detailed in the invention, the nucleic acid cassette is cloned into a vector allowing production of a portion or the entire light or heavy chain sequence fused to all or a portion of a viral coat protein (i.e., creating a fusion protein) and displayed on the surface of a particle or cell. While several types of vectors are available and may be used to practice this invention, phagemid vectors are the preferred vectors for use herein, as they may be constructed with relative ease, and can be readily amplified. Phagemid vectors generally contain a variety of components including promoters, signal sequences, phenotypic selection genes, origin of replication sites, and other necessary components as are known to those of ordinary skill in the art.

When a particular variant amino acid combination is to be expressed, the nucleic acid cassette contains a sequence that is able to encode all or a portion of the heavy or light chain variable domain, and is able to encode the variant amino acid combinations. For production of antibodies containing these variant amino acids or combinations of variant amino acids, as in a library, the nucleic acid cassettes can be inserted into an expression vector containing additional antibody sequence, for example all or portions of the variable or constant domains of the light and heavy chain variable regions. These additional antibody sequences can also be fused to other nucleic acids sequences, such as sequences that encode viral coat proteins and therefore allow production of a fusion protein.

Humanization of murine anti-human STEAP-1 antibody is described herein.

Materials and Methods

Residue numbers are according to Kabat (Kabat *et al., Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Single letter amino acid abbreviations are used. DNA degeneracies are represented using the IUB code (N = A/C/G/T, D = A/G/T, V = A/C/G, B= C/G/T, H= A/C/T, K = G/T, M = A/C, R = A/G, S = G/C, W= A/T, Y = C/T).

Cloning of murine 120 variable domains and generation of a chimeric 120 antibody – Total RNA was extracted from hybridoma cells producing M2-120.545 (designated as "murine 120" or "mu120" herein) using standard methods. The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers to the heavy and light chains. The forward primers were specific for the N-terminal amino acid sequence of the VL and VH regions. Respectively, the LC and HC reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which are highly conserved across species. Amplified VL and VH were cloned into mammalian expression vectors. The polynucleotide sequence of the inserts was determined using routine sequencing methods. The M2-120.545 ("mu 120") VL and VH amino acid sequences are shown in Figures 2A and 2B, respectively.

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Generation of murine 120 chimera – A chimeric anti-STEAP-1 antibody was prepared by fusing the murine 120 variable heavy (VH) and variable light (VL) regions to the constant domains of a human IgG. The resultant antibody is designated "120 chimera," "chimera 120," "chimeric 120 IgG," or "Fc chimera" herein.

Direct hypervariable region grafts onto the acceptor human consensus framework – Variants constructed during the humanization of murine 120 were assessed both as protein in the form of an IgG or as a Fab displayed on phage.

The phagemid used for this work is a monovalent Fab-g3 display vector and consists of two open reading frames under control of the phoA promoter. The first open reading frame consists of the stII signal sequence fused to the VL and CH1 domains of the acceptor light chain and the second consists of the stII signal sequence fused to the VH and CH1 domains of the acceptor heavy chain followed by the minor phage coat protein P3.

The VL and VH domains from murine 120 were aligned with the human VL kappa I (huKI) and human VH subgroup III (huIII) consensus sequences. To make the CDR grafts, hypervariable regions from the murine 120 antibody were grafted into the huKI and huIII acceptor frameworks.

Hypervariable regions from murine 120 antibody (mu120) were engineered into the acceptor human consensus framework to generate the direct CDR-graft (designated as "120 graft," or "graft 120" herein). In the VL domain the following regions were grafted to the human consensus acceptor: positions 24-34 (L1), 50-56 (L2) and 89-97 (L3). In the VH domain, positions 26-35a (H1), 49-65 (H2) and 95-102 (H3) were grafted. The sequences of the light and heavy chain variable regions of the 120 graft are shown in Figures 2A-2B. The CDRs (also designated here as HVRs) are shown in boxes (Figures 2A-2B). These CDR definitions include positions defined by their sequence hypervariability (Kabat ref), their structural location (Chothia ref) and their involvement in antigen-antibody contacts (MacCallum *et al. J. Mol. Biol.* 262: 732-745 (1996)).

The direct-graft variants expressed as a Fab displayed on phage or as an IgG were generated by Kunkel mutagenesis using a separate oligonucleotide for each hypervariable region. Correct clones were assessed by DNA sequencing.

Generation of humanized 120 phage variants – Humanized 120 variants were generated as Fab displayed on phage by Kunkle mutagenesis. A phosphorylated oligonucleotide was added to 300 ng Kunkel template in 50 mM Tris pH 7.5, 10 mM $MgCl_2$ in a final volume of 10 µl. The mixture was annealed at 90 °C for 2 min, 50 °C for 5 min and then cooled on ice. The annealed template was then filled in by adding 0.5 µl 10mM ATP, 0.5 µl 10mM dNTPs (10mM each of dATP, dCTP, dGTP and dTTP), 1 µl 100mM DTT, 1 µl 10X TM buffer (0.5 M Tris pH 7.5, 0.1 M $MgCl_2$), 80 U T4 ligase, and 4 U T7

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polymerase in a total volume of 20 µl for 2 h at room temperature. The filled in and ligated product was then transformed into XL1-blue cells (Stratagene). Correct clones were identified by DNA sequencing.

Correct phage clones were grown in 25 ml of 2YT containing 50 µg/ml carbenacillin and M13/KO7 helper phage (MOI 10) overnight at 37 °C.

Assessment of humanized 120 variants – Humanized variants expressed as IgG were assessed by FACS analysis using Steap1 positive (293 Steap1 NT LB50) and negative (293 vector S408) cell lines. Humanized variants expressed as a Fab displayed on phage were also assessed by FACS analysis. Phage expressing Fab variants were first assessed for their Fab display level using an phage ELISA used to detect a flag-tag fused to the light chain of the Fab. MaxiSorp microtiter plates were coated with anti-gD 1766 at 10 µg/ml in PBS over night and then blocked with Casein Blocker. Phage from culture supernatants were serially diluted in PBST containing 0.5 % BSA in a tissue culture microtiter plate and transferred to the coated wells for 1 h to capture the Fab displaying phage. The plate was washed with PBST and HRP conjugated anti-M13 (Amersham Pharmacia Biotech) was added (1:5000 in PBST containing 0.5 % BSA) for 40 min. The plate was washed with PBST and developed by adding Tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The absorbance at 405 nm was used as an estimate of the Fab display level on the surface of the phage. Phage preparations were normalized for display by dilution. Low displaying phage (e.g. the chimera) were used neat for FACS analysis.

For FACS analysis of phage binding, cells were removed from the plate using 2 mM EDTA, collected in a 15 mL conical bottom tube and pelleted by centrifugation. Cells (5 X 10^5 cells per sample) were re-suspended in 100 µL of phage (normalized by display level) in FACS buffer (1% FBS, PBS with 2 mM EDTA) and incubated for 1-2 hours on ice. Samples were washed twice with FACS buffer by centrifugation. Anti-M13 5G7 control antibody (Genentech, Inc. South San Francisco, CA) was added at 2 µg/mL and incubated on ice for at least 45 minutes. Samples were washed twice with FACS buffer by centrifugation. A 1:200 dilution of anti-mouse PE (R-phycoerythrin goat anti-mouse IgG Fcy Fragment, Jackson Immunoresearch) was added and incubated on ice for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and analyzed by FACS.

For analysis of IgG by FACS, cells were prepared as in the phage FACS. Each IgG was added at 5 µg/mL on ice for 1 hour. Samples were washed twice with FACS buffer by centrifugation and a 1:200 dilution of anti-human PE conjugate (R-phycoerythrin goat anti-human IgG Fcy Fragment, Jackson Immunoresearch) was added for 30 minutes. Samples were again washed twice with FACS buffer by centrifugation and samples were analyzed by FACS.

IgG Production and Affinity Determination -IgG was purified with Protein G affinity chromatography. Affinity determinations were performed by Scatchard analysis on 293 STEAP-1 NT LB50 cells.

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Results and Discussion

Murine 120 variable domain sequences and CDR assignment graft design – The human acceptor framework used for humanization of M2-120.545 is based on the consensus human kappa I VL domain and the human subgroup III consensus VH domain. The VL and VH domains of murine M2-120.545 were each aligned with the human kappa I and subgroup III domains; each complimentarity region (CDR) was identified and grafted into the human acceptor framework to generate a CDR graft that could be displayed as an Fab on phage and expressed as an IgG. The sequences of humanized anti-STEAP-1 antibody version 24 variable regions are shown in Figures 2A and 2B. The 120-graft Fab displayed on phage and 120-graft IgG were tested for binding to exogenous STEAP-1 expressing cells (293 STEAP-1 NT LB50) by FACS analysis. Although the 120-graft IgG bound specifically to the STEAP-1 expressing cells, the FACS signal observed for the 120-graft IgG was smaller than that observed for the chimeric 120 IgG indicating a loss in binding affinity. Phage displaying the 120-graft Fab also generated a FACS signal that was only observed on STEAP-1 expressing cells. This shift was less than that observed for the chimeric 120 IgG. Scatchard analysis of the 120-graft IgG also indicated a significant (approximately 50-fold) loss in binding affinity (KD = 36 nM for the 120v.78; KD = 260 nM for 120 graft).

Humanization of M2-120.545 –Approximately 30 vernier positions that influence CDR conformation and VL:VH domain packing have been identified and changes at these positions between the donor and human frameworks should be considered when humanizing antibodies (Foote, J. and Winter, G., J. Mol. Biol.224(2):487-499 (1992)). An assessment of the alignment of murine M2-120.545 with the consensus human kappa I VL domain and the human subgroup III consensus VH domain revealed sequence differences at 6 key vernier positions in the VH domain: 24, 37, 48, 67, 73, 78 (see Figure 2B). To assess the influence of these positions, murine residues were individually introduced into the human consensus subgroup III VH domain of the Fab on phage. This involved making the following mutations to the 120-graft Fab displayed on phage individually: A24V (120.v24), V37I (120.v37), V48M (120.v48), F67I (120.v67), and L78F (120.v78). N73T was not tested. Each phage variant was normalized by dilution to an equivalent Fab display level determined by titration of an epitope tag fused to the light chain displayed on the phage and then assessed for binding to STEAP-1 by FACS analysis on STEAP-1-expressing cells (293 STEAP-1 NT LB50) and non-expressing (293 vector S408) cells. The term "2°" refers to the secondary antibody in the FACS analysis. The term "α-120" refers to the murine 120 anti-STEAP-1 antibody. The term "α-10H1" refers to a control antibody. The terms "24 Phage," "37 Phage," and the like refer to humanized anti-STEAP-1 variants as disclosed herein displayed on phage. "Ch 120 Phage" refers to the 120 Chimera displayed on phage, and "120.graft Phage" refers to the 120 graft displayed on Phage. (Figure 6). The importance of normalizing phage clones by their Fab display level is illustrated by a FACS analysis of the 120-graft at different phage titers: $7x10^{12}$ phage/ml in Figure 6 and 2×10^{11} phage/ml in Figure 6.

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Once diluted to the lower phage concentration, the 120-graft phage no longer produced an observable FACS shift. Thus normalization of the different phage clones for their display level was important step for assessing their affinity differences for Steap1.

Following normalization for Fab display levels, the variant of the 120-graft containing the additional mutation A24V (120.v24) produced a FACS shift superior to other variants (Figure 6). When expressed as an IgG, 120.v24 produced a similar FACS shift to the chimeric 120 antibody at all concentrations tested. Subsequent Scatchard analysis of 120.v24 indicated a Kd of 2.2 nM for binding to 293 STEAP-1 NT LB50 cells, a two-fold improvement over the 120 chimera and the original murine M2-120.545 (Table 2).

Table 2: Anti-STEAP-1 antibody binding affinity for cell surface STEAP-1 (Kd (nM))

Cell line	**Murine anti-STEAP-1 MAb 120.545 nM**	**120 Chimera**	**Humanized anti-STEAP-1 120v.24**
PC3-PS5.4 (exogenous STEAP-1)	17.5 nM 187,256 sites per cell	9.9 nM 103,204 sites per cell	---
293.LB50 (exogenous STEAP-1)	4.7 nM 301,100 sites per cell	4.9 nM 252,892 sites per cell	2.2 nM 264,172 sites per cell
LNCaP-BR (endogenous STEAP-1)	1.5 nM 37,207 sites per cell	0.9 nM 22,021 sites per cell	---

Binding activity of anti-STEAP-1 naked antibodies, murine 120 and chimera 120 was also tested using FACS analysis. Binding was compared for exogenous STEAP-1 in 293 stable STEAP-1 NT LB50, PC3 stable STEAP-1 PS5.4, and endogenous STEAP-1 in LNCaP cells. The results are also shown in Figs. 7D-7F. NT LB50 cells expressing exogenous human STEAP-1 on the cell surface were prepared by stably transforming 293 cells (ATCC CRL-1573) with human STEAP-1 DNA. PS5.4 cells expressing exogenous human STEAP-1 on the cell surface were prepared by stably transforming PC3 (ATCC CLL-1435) with human STEAP-1 DNA. LNCaP cells (ATCC CRL-1740) express STEAP-1 endogenously.

Example 2: Characterization of anti-STEAP-1 antibodies

Anti-STEAP-1 antibodies (naked antibodies and antibody drug conjugates disclosed herein) were characterized or may be characterized according to standard methods.

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ELISA-based assays: Anti-STEAP-1 antibody screening by ELISA is performed as follows, with all incubations done at room temperature. Test plates (Nunc Immunoplate) were coated for 2 hours with purified STEAP-1 in 50 mM sodium carbonate buffer, pH 9.6, then blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS) for 30 minutes, then washed four times with PBS containing 0.05% Tween 20 (PBST). Test antibody supernatants are added and incubated two hours with shaking, then washed four times with PBST. The plates are developed by adding 100 µl/well of a solution containing 10 mg of o-phenylenediamine dihydrochloride (Sigma, #P8287) and 10 µl of a 30% hydrogen peroxide solution in 25 ml phosphate citrate buffer, pH 5.0, and incubating for 15 minutes. The reaction is stopped by adding 100 µl/well of 2.5 M sulfuric acid. Data is obtained by reading the plates in an automated ELISA plate reader at an absorbance of 490 nm.

Characterization of anti-STEAP-1 binding by Scatchard analysis:

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980) using standard techniques well know in the relevant art. See also Scathcard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

Example 3: Production of Anti-STEAP-1 Antibody Drug Conjugates

Production of anti-STEAP-1 auristatin ADCs - Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies murine 120.545, 120 chimera, 120 graft, and humanized 120 framework variants to the following drug-linker moieties: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE, MC-MMAF, vc-MMAE, and vc-MMAF, which drug and linker moieties and methods of attachment are disclosed herein as well as in WO 2004/010957, published February 5, 2004, WO2006/034488, published September 9, 2005, and in Doronina, S.O. et al., Nature Biotechnol. 21:778-784 (2003), (each of which references is herein incorporated by reference in its entirety). Prior to conjugation, the antibodies were partially reduced with TCEP using standard methods in accordance with the methodology described in WO 2004/010957. The partially reduced antibodies were conjugated to the above drug-linker moieties using standard methods in accordance with the methodology described in Doronina et al. (2003) Nat. Biotechnol. 21:778-784 and US 2005/0238649 A1. Briefly, the partially reduced antibodies were combined with the drug linker moieties to allow conjugation of the moieties to cysteine residues. The conjugation reactions were quenched, and the ADCs were purified. The drug load (average number of drug moieties per antibody) for each ADC was determined by HPLC. As used herein, the linker-drug component of an ADC, " –MC-vc-PAB-MMAE" or "-MC-vc-PAB-MMAF" is sometimes abbreviated as " –vcMMAE" or "-vcMMAF," and the component "-MC-MMAF" is sometimes abbreviated as "MCMMAF" or "mcMMAF."

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Production of anti-STEAP-1 maytansinoid ADCs – Anti-STEAP-1 ADCs were produced by conjugating anti-STEAP-1 antibodies, murine 120, 120 chimera, 120 graft, and humanized 120 framework variants to the linker drug moiety –smcc-DM1. Such conjugation may be performed according to the method disclosed in WO 2005/037992 for conjugation of Herceptin® anti-HER2 antibody.

Example 4: In Vivo Tumor Volume Reduction Assay

To test the efficacy of toxin-conjugated or unconjugated anti-STEAP-1 monoclonal antibodies for the ability to reduce tumor volume in vivo and in vitro, the following protocol was employed.

Mammalian cell lines and human tumor xenografts: 293 is a human immortalized embryonic kidney cell line (ATCC reference CRL1573), PC-3 is a human prostate adenocarcinoma cell line (ATCC reference CRL1435) and LNCaP is a prostate carcinoma cell line (ATCC CRL1740). All cells were grown in 50/50 Dulbecco modified Eagle high glucose medium, Ham's F12 supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, 1% penicillin-streptomycin and cultured at 37°C in 5% CO2. 293 and PC-3 stable cell lines were generated by transfection (Fugene, Roche) with a cytomegalovirus-driven vector encoding either full length STEAP1 (LB50 and PS5.4 respectively) or an empty vector and selected in 400 µg/ml G418 (Geneticin, Life Technologies). Human prostate explant models, LuCAP 77 and LuCAP 35V, were obtained from the University of Seattle.

Expression of exogenous and endogenous STEAP-1 on the cell surface was demonstrated by immunohistohemistry (IHC) and FACS analysis as follows. Sheep and mouse anti-STEAP-1 antibodies (Agensys, Inc., Santa Monica, CA) were generated against an intracellular amino-terminal peptide of STEAP-1 (see Hubert, R.S., Vivanco, I. et al., PNAS 25:14523-14528 (1999)). Monoclonal antibodies against the extracellular domains of STEAP-1 (Agensys, Inc.) were generated by immunization of mice with 293T cells transiently transfected with STEAP-1. For IHC analysis, the primary sheep anti-STEAP-1 antibody was used for detection. For FACS analysis, cells were grown to 90% confluence and removed from plates using 2 mM EDTA in PBS. Cells were washed and resuspended in FACS buffer (PBS with 1% BSA) and incubated for 60 minutes with anti-STEAP1 antibodies at room temperature followed by 60 minutes with the appropriate secondary antibody conjugated to phycoerythrin. Analysis was performed on FACSscan (BD Biosciences). For immunoflourescence, cells were grown in chamber slides overnight and then incubated with primary antibody at 37°C for 60 minutes. Cells were fixed in paraformaldehyde , blocked in 1% BSA and incubated with the appropriate secondary antibody conjugated to flourescin.

In vivo prostate cancer xenograft models were used to test the efficacy of anti-STEAP-1 ADCs. These models included human cell line LNCaP (ATCC CRL-1740 or Southern Research Institute, Birmingham, AL). Prostate explant models included LuCaP 77 and LuCaP35V (University of Washington, Seattle, WA). Each prostate explant model was maintained by serial transplanting in castrated (androgen independent model, LuCAP 35V) or uncastrated (androgen dependent model, LuCAP

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77), male SCID-beige mice from Charles River Lab. The uncastrated mice received a testosterone pellet prior to implantation, while castration was done at least two weeks prior to tumor implantation to allow testosterone levels to nadir. When donor mice had tumors of between 800-1000 mm^3, tumor tissue were aseptically removed and dissected into small implantable sized pieces (approximately 20 mm^3) for study animals. The tumor is placed into a pocket at the implantation site and the skin is closed using wound clips. For the LNCaP cell line model, in vitro grown LNCaP cells were injected subcutaneously at 8-10 million cells per mouse in 50% matrigel into male SCID-beige mice which had received a testosterone pellet. When mean tumor size reached 100-200 mm^3, animals were randomly grouped in ten groups of ten mice each and given a single IV administration of test antibody ADC or control antibody (naked or control). In some experiments, multiple doses of test or control antibody were administered (see Figures 8A, 9, and 10). In some experiments, a single dose of test and control antibody were administered as seen in Figures 8B and 11. Where the prostate explant model was LuCap 77, a testosterone pellet was implanted in the mice approximately 3-7 days before transplantation of exogenous tumor. Tumors were measured twice per week for 4 weeks, then once or twice per week for the remainder of the study or once per week throughout the study. A significantly lower tumor volume in test animals over time was considered to an indication of efficacy. In some cases, tumor volume decreased significantly from the initial volume and remained low throughout the study. Results are plotted in Figures 8-11.

Anti-STEAP-1 auristatin drug conjugates reduce prostate tumor volume in vivo

Administration of murine anti-STEAP-1 120-MC-vc-PAB-MMAE at 3 mg/kg was efficacious in a prostate tumor (LNCaP-Ner cells) xenograft model. PBS and anti-gp120-MC-vc-PAB-MMAE (3 mg/kg) were used as controls. Doses were administered on days 0, 7, and 14. See Figure 8A.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg), and anti-STEAP-1 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) to SCID beige mice transplanted with LNCap-Ner tumor (treated with a testosterone pellet as described herein) was shown to be efficacious. Vehicle, anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (12 mg/kg) were used as controls. Doses were administered on the days indicated in Figure 8. The results are plotted in Figure 8B.

Administration of anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) and anti-STEAP-1 120 chimera-MC-MMAF (6 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID-beige mice transplanted with LNCaP cells. Three doses at approximately days 15, 25, and 30 at 3 mg/kg (anti-STEAP-vcMMAE) or 6 mg/kg (anti-STEAP-mcMMAF) were administered to the mice. Control anti-ragweed-MC-vc-PAB-MMAE (3 mg/kg) and anti-ragweed-MC-MMAF (6 mg/kg) were used. See Figure 9.

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Administration of humanized anti-STEAP-1 antibody 120 chimera-MC-vc-PAB-MMAE (3 mg/kg) was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 77 cells. Controls were vehicle and anti-ragweed-MC-vc-PAB-MMAE. Three doses at 3 mg/kg of test and control antibodies were administered. See Figure 10.

Administration of humanized anti-STEAP-1 antibody 120v.24-MC-vc-PAB-MMAE at 3 mg/kg, anti-STEAP-1 antibody 120v.24-MC-MMAF at 6 mg/kg and 12 mg/kg to castrated SCID-beige mice transplanted with LuCap35V prostate tumor was shown to be efficacious relative to controls. Drug load was 3.1 per antibody. Control antibodies were anti-ragweed-MC-MMAF administered at 12 mg/kg, and anti-gp120-MC-vc-PAB-MMAE administered at 6 mg/kg. See Figure 11.

Anti-STEAP-1 auristatin drug conjugates reduce prostate tumor volume in vitro

In vitro cell killing assays were performed to assess the effectiveness of anti-STEAP-1 drug conjugates to inhibit growth and/or kill cells expressing STEAP-1. Briefly, cells expressing STEAP-1 were plated at approximately 2,000 cells/well in a 96-well plate and treated 24 hours later in duplicate with antibody drug conjugate. Plates were incubated for 5-7 days at 37°C and developed with CellTiter-Glo® luminescent cell viability assay kit (Promega, Madison, WI, USA). Test cells included PS5.4 (PC3 cells expressing exogenous STEAP-1), LB50 (293 cells expressing exogenous STEAP-1), PC3 cells transfected with vector alone, 293 cells transfected with vector alone, and LNCaP cells expressing endogenous STEAP-1. Tested antibody drug conjugates included control antibody-MC-MMAF, control antibody-vc-MMAE, anti-STEAP-1 antibody 120 chimera-vc-MMAE, anti-STEAP-1 antibody 120 chimera-MC-MMAF (two different lots of material), and anti-STEAP-1 antibody chimera-vc-MMAF. The results are shown in Figure 14A-E.

Example 5: Preparation of Cysteine Engineered Anti-STEAP-1 Antibodies for Conjugation By Reduction and Reoxidation

Full length, cysteine engineered anti-STEAP-1 monoclonal antibodies (ThioMabs) expressed in CHO cells are dissolved in 500mM sodium borate and 500 mM sodium chloride at about pH 8.0 and reduced with about a 50-100 fold excess of 1 mM TCEP (tris(2-carboxyethyl)phosphine hydrochloride (Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA) for about 1-2 hrs at 37 °C. The reduced ThioMab is diluted and loaded onto a HiTrap S column in 10 mM sodium acetate, pH 5, and eluted with PBS containing 0.3M sodium chloride. The eluted reduced ThioMab is treated with 2 mM dehydroascorbic acid (dhAA) at pH 7 for 3 hours, or 2 mM aqueous copper sulfate ($CuSO_4$) at room temperature overnight. Ambient air oxidation may also be effective. The buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the

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thiol concentration by reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm.

Example 6: Preparation of Cysteine Engineered Anti-STEAP-1 Antibody Drug Conjugates By Conjugation of Cysteine Engineered Anti-STEAP-1 Antibodies and Drug-linker Intermediates

After the reduction and reoxidation procedures of Example 5, the cysteine engineered anti-STEAP antibody is dissolved in PBS (phosphate buffered saline) buffer and chilled on ice. About 1.5 molar equivalents relative to engineered cysteines per antibody of an auristatin drug linker intermediate, such as MC-MMAE (maleimidocaproyl-monomethyl auristatin E), MC-MMAF, MC-val-cit-PAB-MMAE, or MC-val-cit-PAB-MMAF, with a thiol-reactive functional group such as maleimido, is dissolved in DMSO, diluted in acetonitrile and water, and added to the chilled reduced, reoxidized antibody in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and the cysteine engineered anti-STEAP-1 antibody drug conjugate is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μm filters under sterile conditions, and frozen for storage.

By the procedure above, the following cysteine engineered anti-STEAP-1 antibody drug conjugates were prepared:

thio human120-MC-MMAF by conjugation of V211C thio hu 120 and MC-MMAF;

thio human120-MC-MMAF by conjugation of A125C thio hu 120 and MC-MMAF;

thio human120-MC-val-cit-PAB-MMAE by conjugation of V211C thio hu 120 and MC-val-cit-PAB-MMAE; and

thio human120-MC-val-cit-PAB-MMAE by conjugation of A125C thio hu 120 and MC-val-cit-PAB-MMAE.

Example 7: Characterization of cysteine engineered anti-STEAP-1 antibodies

The cysteine engineered anti-STEAP-1 antibody drug conjugates (TDCs) prepared as described above were assayed to confirm that they retained the activity of the parent antibody in vitro. The anti-STEAP-1 TDCs thio-human120-vc-PAB-MMAE (LCV211C) (abbreviated as huSteap1 TDC (L211C) vcE and thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 TDC (HCA125C) vcE) were assessed for binding to STEAP-1 by FACS analysis on STEAP-1-expressing cells (293 STEAP-1 NT LB50) and non-expressing (293 vector S408) cells. The term "2nd only" refers to the secondary antibody in the FACS analysis. The TDC control (vcE) and ADC std control (vcE) are control antibody thio and non-thio vc-PAB-MMAE drug conjugates, respectively. The huSteap1 ADC (std) is a vc-PAB-MMAE drug conjgate derived from the parent human anti-STEAP-1 antibody. As shown, the TDCs produced FACS shifts similar to that of the parent huSteap1 ADC.

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In vitro cell killing assays were also performed, to assess the effectiveness of the cysteine engineered anti-STEAP-1 antibody drug conjugates to inhibit growth and/or kill cells expressing STEAP-1. Briefly, cells expressing STEAP-1 were plated at approximately 2,000 cells/well in a 96-well plate and treated 24 hours later in duplicate with antibody drug conjugate. Plates were incubated for 5-7 days at 37°C and developed with CellTiter-Glo® luminescent cell viability assay kit (Promega, Madison, WI, USA). Test cells included PS5.4 (PC3 cells expressing exogenous STEAP-1), LB50 (293 cells expressing exogenous STEAP-1), and LNCaP cells expressing endogenous STEAP-1. Tested antibody drug conjugates included control antibody-vc-MMAE (ADC std control (vcE)), control thio antibody-vc-MMAE (TDC control (vcE)), the anti-STEAP-1 TDCs thio-human120-vc-PAB-MMAE (LCV211C) (abbreviated as huSteap1 TDC (L211C) vcE and thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 TDC (HCA125C) vcE), and huSteap1 ADC (std), a vc-PAB-MMAE drug conjgate derived from the parent human anti-STEAP-1 antibody. As shown in Figures 19A-C, the anti-STEAP-1 TCDs retain the activyt of the parent ADC in vitro..

Example 8: In Vivo Tumor Volume Reduction Assays for CysteineEngineered Anti-STEAP-1 Antibody Drug Conjugates

In vivo prostate cancer xenograft models were used to test the efficacy of cysteine-engineeered anti-STEAP-1 ADCs. These models and the test protocols employed correspond to those described in Example 4.

Administration of the anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 HC TDC vcE) (3 mg/kg) to SCID beige mice transplanted with LNCap-Ner tumor (treated with a testosterone pellet as described herein) was shown to be efficacious. Vehicle (PBS), control antibody-vc-MMAE (ADC std ctrl vcE) and control thio antibody-vc-MMAE (TDC HC ctrl vcE) were used as controls. The effect of the anti-STEAP-1 TDC was also compared to human anti-STEAP-1 antibody 120-MC-vc-PAB-MMAE (hu Steap1 std ADC vcE) as a positive control. A single dose was administered at day 0. All antibodies were administered at 3 mg/kg. The results are plotted in Figure 20.

Figure 21 shows that administration of anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 HC TDC vcE) at 3 mg/kg and anti-STEAP-1 TDC thio-human120-MC-MMAF (HCA125C) (abbreviated as huSteap1 HC TDC mcF) at 1, 3 or 6 mg/kg was shown to be efficacious in a prostate cancer xenograft model of SCID-beige mice transplanted with LNCaP cells. Single doses at day 0 at 0.3, 1 or 3 mg/kg (huSteap1 HC TDC vcE) or 1, 3 or 6 mg/kg (huSteap1 HC TDC mcF) were administered to the mice. Vehicle (PBS), control antibody-vc-MMAE (ADC std ctrl vcE) and control thio antibody-vc-MMAE (TDC HC ctrl vcE) were used as controls.

Figure 22 shows that administration of anti-STEAP-1 TDC thio-human120-vc-PAB-MMAE (HCA125C) (abbreviated as huSteap1 HC TDC vcE) at 3 mg/kg and anti-STEAP-1 TDC thio-human120-

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MC-MMAF (HCA125C) (abbreviated as huSteap1 HC TDC mcF) at 3 or 6 mg/kg was shown to be efficacious in a prostate cancer xenograft model of SCID beige male mice (androgen dependent) transplanted with LuCap 35V cells. Single doses at day 0 at 0.3, 1 or 3 mg/kg (huSteap1 HC TDC vcE) or 1, 3 or 6 mg/kg (huSteap1 HC TDC mcF) were administered to the mice. Vehicle (PBS), control antibody-vc-MMAE (ADC std ctrl vcE) and control thio antibody-vc-MMAE (TDC HC ctrl vcE) were used as controls.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

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WHAT IS CLAIMED IS:

1. An antibody that binds to STEAP-1, wherein the antibody comprises at least one, two, or three heavy chain (HC) hypervariable regions (HVRs) selected from:
(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

2. The antibody of claim 1, further comprising comprising at least one, two, or three HC framework regions (FRs) selected from:
(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

3. The antibody of claim 1, further comprising a light chain (LC) of SEQ ID NO:6.

4. The antibody of claim 2, further comprising a light chain (LC) of SEQ ID NO:6.

5. The antibody of claim 1, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

6. The antibody of claim 2, wherein the HC has at least 90% sequence identity to SEQ ID NOs:9 or 10.

7. The antibody of claim 1, wherein the antibody is humanized.

8. The antibody of claim 2, wherein the antibody is humanized.

9. The antibody of claim 1, wherein the STEAP-1 is mammalian STEAP-1.

10. The antibody of claim 9, wherein the STEAP-1 is selected from rodent STEAP-1 and primate STEAP-1.

11. The antibody of claim 31, wherein the STEAP-1 is human STEAP-1.

12. A polynucleotide encoding a HC comprising at least one, two, or three HC-HVRs selected from:
(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15; and

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(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16.

13. A polynucleotide of claim 12 comprising at least one, two, three, or four HC-FRs selected from:

(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25

(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;

(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;

(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;

(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;

(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;

(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and

(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

14. A vector comprising the polynucleotide of claim 12.

15. A host cell comprising the vector of claim 12.

16. The host cell of claim 15, wherein the host cell is a bacteria, yeast, insect, or eukaryotic cell.

17. The host cell of claim 42, wherein the host cell is a CHO cell.

18. A method of making an anti-STEAP-1 antibody, wherein the method comprises a) culturing the host cell of claim 15 under conditions suitable for expression of the polynucleotide encoding the antibody, and b) isolating the antibody.

19. The antibody of claim 1, wherein the STEAP-1 is expressed on the surface of a cell.

20. The antibody of claim 19, wherein the cell is a prostate, lung, colon, bladder, or ovary cell or a Ewing's sarcoma cell.

21. The antibody of claim 20, wherein the cell is associated with a proliferative disorder.

22. The antibody of claim 21, wherein the disorder is cancer.

23. The antibody of claim 22, wherein the cancer is metastasis.

24. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

25. The antibody of claim 24, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

26. The antibody of claim 60, wherein the antibody is humanized.

27. The antibody of claim 60, wherein the antibody is human.

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28. A method of detecting the presence of STEAP-1 in a biological sample, the method comprising contacting the biological sample with an antibody of claim 1 under conditions permissive for binding of the antibody to STEAP-1, and detecting whether a complex is formed between the antibody and STEAP-1.

29. The method of claim 28, wherein the biological sample is from a patient suspected of having a prostate, lung, colon, bladder, ovarian cell or Ewing's sarcoma cell proliferative disorder.

30. An immunoconjugate comprising an antibody of claim 1 covalently attached to a cytotoxic agent.

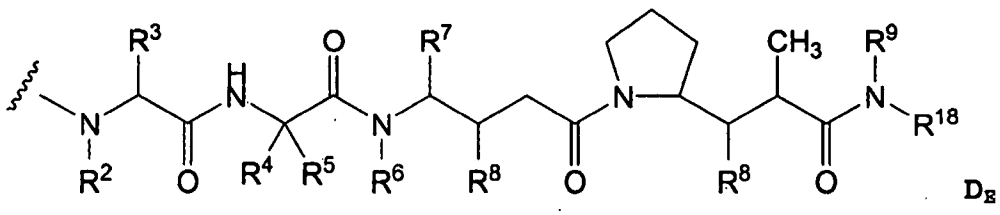
31. The immunoconjugate of claim 30, wherein the cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

32. The immunoconjugate of claim 31, the immunoconjugate having the formula Ab-(L-D)p, wherein:
(a) Ab is the antibody of claim 1;
(b) L is a linker;
(c) D is a drug moiety.

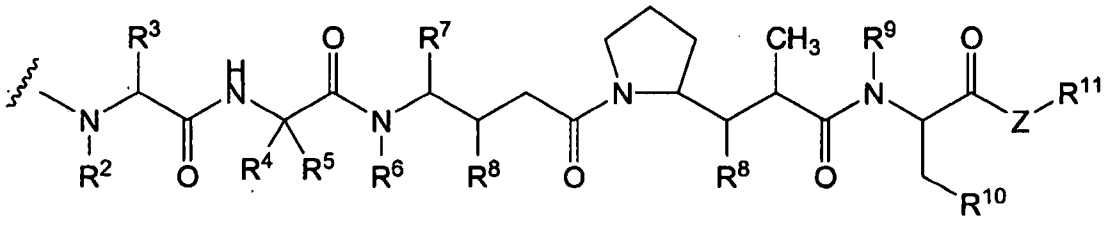
33. The immunoconjugate of claim 32, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB), and 6-maleimidocaproyl-valine-citrulline-p-aminobenyloxycarbonyl (MC-vc-PAB).

34. The immunoconjugate of claim 33, wherein D is selected from an auristatin and dolostantin.

35. The immunoconjugate of claim 34, wherein D is a drug moiety of formula D_E or D_F:



D_E



D_F

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and wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; R^{10} is aryl; Z is –O– or –NH–; R^{11} is H, C_1-C_8 alkyl, or $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-CH_3$; and R^{18} is $-C(R^8)_2-C(R^8)_2-$aryl; and

(d) p ranges from about 1 to 8.

36. The immunoconjugate of claim 32, having in vitro or in vivo cell killing activity.

37. The immunoconjugate of claim 32, wherein the linker is attached to the antibody through a thiol group on the antibody.

38. The immunoconjugate of claim 32, wherein the linker is cleavable by a protease.

39. The immunoconjugate of claim 32, wherein the linker comprises a val-cit dipeptide.

40. The immunoconjugate of claim 32, wherein the linker comprises a p-aminobenzyl unit.

41. The immunoconjugate of claim 32, wherein the linker comprises 6-maleimidocaproyl.

42. The immunoconjugate of claim 32, wherein the drug is selected from MMAE and MMAF.

43. The immunoconjugate of claim 42, wherein the drug is MMAE.

44. The immunoconjugate of claim 42, wherein the drug is MMAF.

45. The immunoconjugate of claim 32, wherein the immunoconjugate has the formula
Ab-(L-MMAE)p,
wherein L is a linker and p ranges from 2 to 5.

46. The immunoconjugate of claim 45, wherein L comprises val-cit.

47. The immunoconjugate of claim 45, wherein L comprises MC.

48. The immunoconjugate of claim 45, wherein L comprises PAB.

49. The immunoconjugate of claim 45, wherein L comprises MC-vc-PAB.

50. The immunoconjugate of claim 45, wherein the immunoconjugate has the formula
Ab-(L-MMAF)p,
wherein L is a linker and p ranges from 2 to 5.

51. The immunoconjugate of claim 50, wherein L comprises val-cit.

52. The immunoconjugate of claim 50, wherein L comprises MC.

53. The immunoconjugate of claim 50, wherein L comprises PAB.

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54. The immunoconjugate of claim 50, wherein L comprises MC-vc-PAB.

55. The immunoconjugate of claim 50, wherein the immunoconjugate has the formula
Ab-(L-MMAF)p,
wherein L is a linker and p ranges from 2 to 5.

56. The immunoconjugate of claim 32, wherein D is a maytansinoid.

57. The immunoconjugate of claim 56, wherein D is selected from DM1, DM3, and DM4.

58. The immunoconjugate of claim 56, having in vitro or in vivo cell killing activity.

59. The immunoconjugate of claim 56, wherein the linker is attached to the antibody through a thiol group on the antibody.

60. The immunoconjugate of claim 56, wherein the linker, L, is selected from N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

61. The immunoconjugate of claim 60, wherein the drug is DM1.

62. The immunoconjugate of claim 62, wherein L comprises a SPP.

63. The immunoconjugate of claim 61, wherein L comprises SMCC.

64. The immunoconjugate of claim 61, wherein p is from 2-6.

65. The immunoconjugate of claim 64, wherein p is from 3-5.

66. A pharmaceutical composition comprising the immunoconjugate of claim 32 and a pharmaceutically acceptable carrier.

67. A method of treating a cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 66.

68. The method of claim 67, wherein the cell proliferative disorder is selected from prostate, lung, colon, bladder, ovary, and Ewing's sarcoma cell proliferative disorder.

69. A method of inhibiting prostate, lung, colon, bladder, ovary, Ewing's sarcoma cell proliferation comprising exposing a cell to an immunoconjugate of claim 32 under conditions permissive for binding of the immunoconjugate to STEAP-1.

70. The method of claim 69, wherein the prostate, lung, colon, bladder, or ovary cell or Ewing's sarcoma cell is a xenograft.

71. The method of claim 69, wherein the exposing takes place in vitro.

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72. The method of claim 69, wherein the exposing takes place in vivo.

73. The antibody of claim 1, wherein the antibody is a cysteine engineered antibody comprising one or more free cysteine amino acids having a thiol reactivity value in the range of 0.6 to 1.0, wherein the cysteine engineered antibody is prepared by a process comprising replacing one or more amino acid residues of a parent antibody by cysteine.

74. The cysteine engineered antibody of claim 73, wherein the cysteine engineered antibody is more reactive than the parent antibody with a thio-reactive reagent.

75. The cysteine engineered antibody of claim 73 wherein the process further comprises determining the thiol reactivity of the cysteine engineered antibody by reacting the cysteine engineered antibody with a thiol-reactive reagent;

wherein the cysteine engineered antibody is more reactive than the parent antibody with the thiol-reactive reagent.

76. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a light chain.

77. The cysteine engineered antibody of claim 73 wherein the one or more free cysteine amino acid residues are located in a heavy chain.

78. The cysteine engineered antibody of claim 73, wherein the antibody is an immunoconjugate comprising the cysteine engineered antibody covalently attached to a cytotoxic agent.

79. The cysteine engineered antibody of claim 78, wherein the cytotoxic agent is selected fom a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

80. The cysteine engineered antibody of claim 73 wherein the antibody is covalently attached to a capture label, a detection label, or a solid support.

81. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a biotin capture label.

82. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a fluorescent dye detection label.

83. The cysteine engineered antibody of claim 82 wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

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84. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a radionuclide detection label selected from ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi.

85. The cysteine engineered antibody of claim 80 wherein the antibody is covalently attached to a chelating ligand that chelates a detectable label.

86. The cysteine engineered antibody of claim 80 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

87. The immunoconjugate of claim 32, wherein the linker is attached to the antbody through a thiol group on the antibody.

88. The antibody of claim 1 comprising an albumin binding peptide.

89. The antibody of claim 88, wherein the albumin binding peptide is selected from SEQ ID NOs:80-84.

90. The method of claim 67, further comprising administering to the individual an effective amount of at least one additional therapeutic compound selected from a chemotherapeutic acid, a radionuclide, CHOP, a naked antibody, antibody drug conjugate, and an immunoconjugate.

91. The method of claim 90, wherein the antibody, ADC, and immunoconjugate bind to an antigen expressed in proliferating cells.

92. The method of claim 91, wherein the proliferating cells are from prostate, lung, colon, bladder, ovary, or Ewing's sarcoma.

93. A cysteine engineered anti-STEAP-1 antibody comprising one or more free cysteine amino acids wherein the cysteine engineered anti-STEAP-1 antibody binds to a STEAP-1 polypeptide and is prepared by a process comprising replacing one or more amino acid residues of a parent anti-STEAP-1 antibody by cysteine wherein the parent antibody comprises at least one HVR sequence selected from:
(a) an HVR-L1 sequence of SEQ ID NO:11;
(b) an HVR-L2 sequence of SEQ ID NO:12;
(c) an HVR-L3 sequence of SEQ ID NO:11;
(d) an HVR-H1 sequence of SEQ ID NO:14;
(e) an HVR-H2 sequence of SEQ ID NO:15; and
(f) aan HVR-H3 sequence of SEQ ID NO:16.

94. The cysteine engineered antibody of claim 1 wherein the parent anti-STEAP-1 antibody comprises a variable heavy chain sequence selected from any one of SEQ ID NOS:9-10.

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95. The cysteine engineered antibody of claim 1 wherein the parent anti-STEAP-1 antibody comprises a variable light chain sequence of SEQ ID NO:6.

96. The cysteine engineered antibody of claim 1 wherein the one or more free cysteine amino acid residues are located in a light chain.

97. The cysteine engineered antibody of claim 4 comprising one or more sequences selected from:
(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:25
(2) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:22;
(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:75;
(4) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:76;
(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:23;
(6) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:78;
(7) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:79; and
(8) an HC-FR4 comprising the amino acid sequence of SEQ ID NO:24.

98. The cysteine engineered antibody of claim 1 wherein the one or more free cysteine amino acid residues are located in a heavy chain.

99. The cysteine engineered antibody of claim 6 comprising one or more sequences selected from:
(1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:17
(2) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:18;
(3) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:19;and
(4) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:20.

100. The cysteine engineered antibody of claim 93 comprising a heavy chain sequence comprising SEQ ID NO:9.

101. The cysteine engineered antibody of claim 93 comprising a heavy chain sequence comprising SEQ ID NO:10.

102. The cysteine engineered antibody of claim 93 comprising a light chain sequence comprising SEQ ID NO:6.

103. The cysteine engineered antibody of claim 93 wherein the parent anti-STEAP-1 antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

104. The cysteine engineered antibody of claim 93 which is an antibody fragment.

105. The cysteine engineered antibody of claim 104 wherein the antibody fragment is a Fab fragment.

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106. The cysteine engineered antibody of claim 93 which is selected from a chimeric antibody, a human antibody, or a humanized antibody.

107. The cysteine engineered antibody of claim 93 which is produced in bacteria.

108. The cysteine engineered antibody of claim 93 which is produced in CHO cells.

109. A method of determining the presence of a STEAP-1 protein in a sample suspected of containing said protein, said method comprising exposing said sample to a cysteine engineered antibody of claim 93 and determining binding of said antibody to said STEAP-1 protein in said sample, wherein binding of the antibody to said protein is indicative of the presence of said protein in said sample.

110. The method of Claim 109 wherein said sample comprises a cell suspected of expressing said STEAP-1 protein.

111. The method of Claim 109 wherein said cell is a prostate, lung, colon, bladder or ovary cancer cell, or a Ewing's sarcoma cell.

112. The method of Claim 109 wherein the antibody is covalently attached to a label selected from a fluorescent dye, a radioisotope, biotin, or a metal-complexing ligand.

113. A pharmaceutical formulation comprising the anti-STEAP-1 antibody of claim 93, and a pharmaceutically acceptable diluent, carrier or excipient.

114. The cysteine engineered antibody of claim 93 wherein the antibody is covalently attached to an auristatin drug moiety whereby an antibody drug conjugate is formed.

115. The antibody-drug conjugate of claim 114 comprising a cysteine engineered antibody (Ab), and an auristatin drug moiety (D) wherein the cysteine engineered antibody is attached through one or more free cysteine amino acids by a linker moiety (L) to D; the compound having Formula I:

$$Ab-(L-D)_p \qquad \mathbf{I}$$

where p is 1, 2, 3, or 4.

116. The antibody-drug conjugate compound of claim 115 wherein p is 2.

117. The antibody-drug conjugate compound of claim 115 wherein L has the formula:

$$-A_a-W_w-Y_y-$$

where:

A is a Stretcher unit covalently attached to a cysteine thiol of the cysteine engineered antibody (Ab);

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a is 0 or 1;

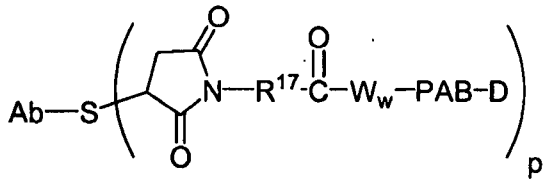
each W is independently an Amino Acid unit;

w is an integer ranging from 0 to 12;

Y is a Spacer unit covalently attached to the drug moiety; and

y is 0, 1 or 2.

118. The antibody-drug conjugate compound of claim 117 having the formula:

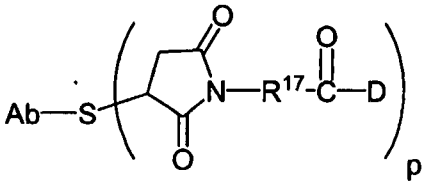


where PAB is para-aminobenzylcarbamoyl, and R^{17} is a divalent radical selected from $(CH_2)_r$, C_3-C_8 carbocyclyl, O–$(CH_2)_r$, arylene, $(CH_2)_r$–arylene, –arylene–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbocyclyl), (C_3-C_8 carbocyclyl)–$(CH_2)_r$, C_3-C_8 heterocyclyl, $(CH_2)_r$–(C_3-C_8 heterocyclyl), –(C_3-C_8 heterocyclyl)–$(CH_2)_r$–, –$(CH_2)_rC(O)NR^b(CH_2)_r$–, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, –$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$–, –$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$–$CH_2$–, –$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$–$CH_2$–, and –$(CH_2CH_2O)_rC(O)NR^b(CH_2)_r$– ; where R^b is H, C_1-C_6 alkyl, phenyl, or benzyl; and r is independently an integer ranging from 1 to 10.

119. The antibody-drug conjugate compound of claim 117 wherein W_w is valine-citrulline.

120. The antibody-drug conjugate compound of claim 117 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

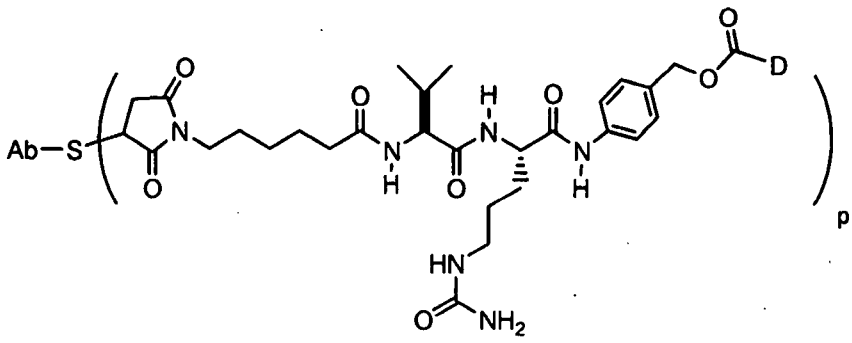
121. The antibody-drug conjugate compound of claim 117 having the formula:



122. The antibody-drug conjugate compound of claim 121 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

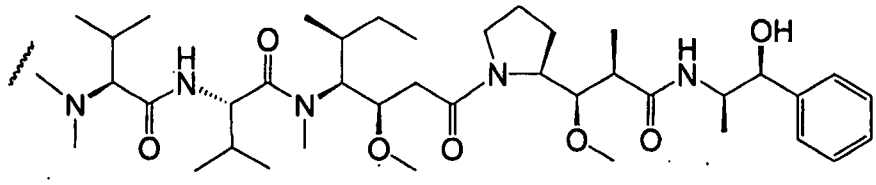
123. The antibody-drug conjugate compound of claim 117 having the formula:

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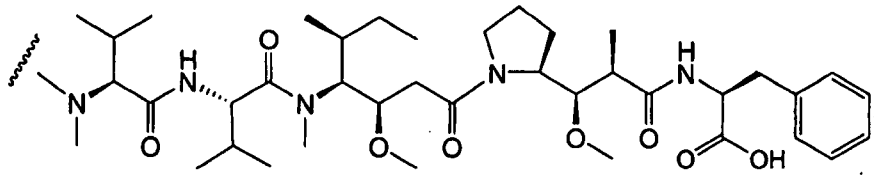
124. The antibody-drug conjugate compound of claim 115 wherein L is SMCC or BMPEO.

125. The antibody-drug conjugate compound of claim 115 wherein D is MMAE, having the structure:



where the wavy line indicates the attachment site to the linker L.

126. The antibody-drug conjugate compound of claim 115 wherein D is MMAF, having the structure:



where the wavy line indicates the attachment site to the linker L.

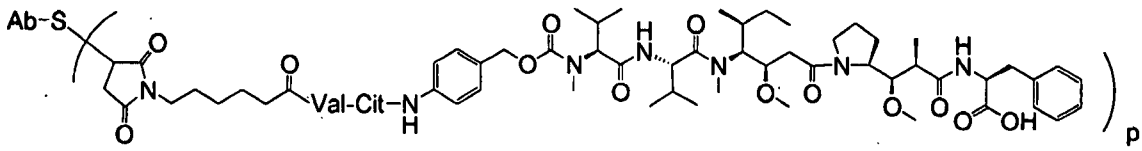
127. The antibody-drug conjugate compound of claim 114 wherein the parent anti-STEAP-1 antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

128. The antibody-drug conjugate compound of claim 114 wherein the parent anti-STEAP-1 antibody is an antibody fragment.

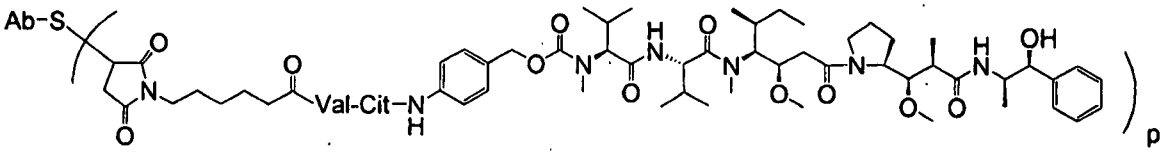
129. The antibody-drug conjugate compound of claim 128 wherein the antibody fragment is a Fab fragment.

130. An antibody-drug conjugate compound selected from the structures:

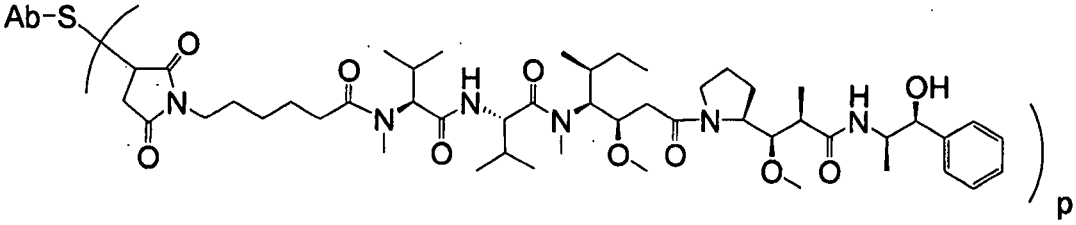
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Ab-MC-vc-PAB-MMAF

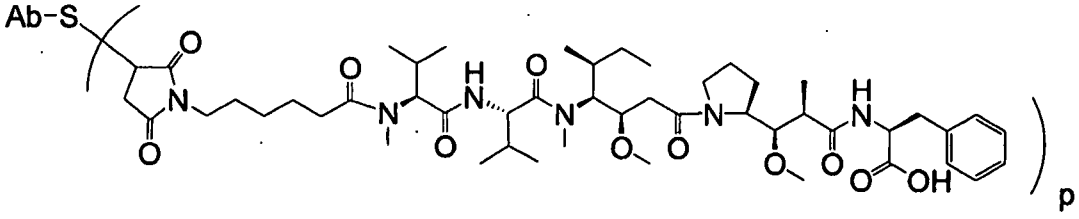


Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE

and



Ab-MC-MMAF

wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or 4; and Ab is a cysteine engineered anti-STEAP-1 antibody of claim 93.

131. The antibody drug conjugate of claim 114 wherein the auristatin is MMAE or MMAF.

132. The antibody drug conjugate of claim 115 wherein L is MC-val-cit-PAB or MC.

133. The antibody drug conjugate of claim 115 wherein L is SMCC, SPP, or BMPEO.

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134. An assay for detecting cancer cells comprising:

(a) exposing cells to an antibody-drug conjugate compound of claim 114; and

(b) determining the extent of binding of the antibody-drug conjugate compound to the cells.

135. The assay of claim 134 wherein the cells are prostate, lung, colon, bladder, ovarian or Ewing's sarcoma tumor cells.

136. A method of inhibiting cellular proliferation comprising treating mammalian tumor cells in a cell culture medium with an antibody-drug conjugate compound of claim 114, whereby proliferation of the tumor cells is inhibited.

137. The method of claim 136 wherein the mammalian tumor cells are prostate, lung, colon, bladder, ovarian or Ewing's sarcoma tumor cells.

138. A pharmaceutical formulation comprising the antibody drug conjugate of claim 114, and a pharmaceutically acceptable diluent, carrier or excipient.

139. The pharmaceutical formulation of claim 138 further comprising a therapeutically effective amount of a chemotherapeutic agent selected from letrozole, oxaliplatin, doxetaxel, 5-FU, lapatinib, capecitabine, leucovorin, and gemcitabine.

140. A method of treating cancer comprising administering to a patient the pharmaceutical formulation of claim 138.

141. The method of claim 140 wherein the cancer is selected from the group consisting of prostate cancer, lung cancer, colon cancer, baldder cancer, ovarian cancer and Ewing's sarcoma.

142. The method of claim 140 wherein the patient is administered a chemotherapeutic agent in combination with the antibody-drug conjugate compound, where the chemotherapeutic agent is selected from letrozole, cisplatin, carboplatin, taxol, paclitaxel, oxaliplatin, doxetaxel, 5-FU, leucovorin, lapatinib, and gemcitabine.

143. An article of manufacture comprising

the pharmaceutical formulation of claim 138;

a container; and

a package insert or label indicating that the compound can be used to treat cancer characterized by the overexpression of a STEAP-1 polypeptide.

144. The article of manufacture of claim 143 wherein the cancer is prostate cancer, lung cancer, colon cancer, bladder cancer, ovarian cancer or Ewing's sarcoma tumor

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145. A method for making an antibody drug conjugate compound comprising a cysteine engineered anti-STEAP-1 antibody (Ab) of claim 1, and an auristatin drug moiety (D) wherein the cysteine engineered antibody is attached through the one or more engineered cysteine amino acids by a linker moiety (L) to D; the compound having Formula I:

$$Ab-(L-D)_p \qquad I$$

where p is 1, 2, 3, or 4; the method comprising the steps of:

(a) reacting an engineered cysteine group of the cysteine engineered antibody with a linker reagent to form antibody-linker intermediate Ab-L; and

(b) reacting Ab-L with an activated drug moiety D; whereby the antibody-drug conjugate is formed;

or comprising the steps of:

(c) reacting a nucleophilic group of a drug moiety with a linker reagent to form drug-linker intermediate D-L; and

(d) reacting D-L with an engineered cysteine group of the cysteine engineered antibody; whereby the antibody-drug conjugate is formed.

146. The method of claim 145 further comprising the step of expressing the cysteine engineered antibody in chinese hamster ovary (CHO) cells.

147. The method of claim 146 further comprising the step of treating the expressed cysteine engineered antibody with a reducing agent.

148. The method of claim 147 wherein the reducing agent is selected from TCEP and DTT.

149. The method of claim 147 further comprising the step of treating the expressed cysteine engineered antibody with an oxidizing agent, after treating with the reducing agent.

150. The method of claim 149 wherein the oxidizing agent is selected from copper sulfate, dehydroascorbic acid, and air.

151. An antibody that binds to STEAP-1, wherein the antibody comprises (a) an HVR-L1 comprising an amino acid sequence of SEQ ID NO:11, and (b) at least one, two, three, four, or five HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:14;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:16;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

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(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:13.

152. The antibody of claim 151 wherein the antibody further comprises a cysteine at one or more positions selected from 15, 43, 110, 144, 168 and 205 of the light chain according to Kabat numbering convention and 41, 88, 115, 118, 120, 171, 172, 282, 375, and 400 of the heavy chain according to EU numbering convention.

153. The antibody of claim 152, wherein a cysteine is at position 205 of the light chain.

154. The antibody of claim 152, wherein a cysteine is at position 118 of the heavy chain.

155. The antibody of claim 152, wherein a cysteine is at position 400 of the heavy chain.

156. The antibody of claim 152 wherein the antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

157. The antibody of claim 152 which is an antibody fragment.

158. The antibody of claim 157 wherein the antibody fragment is a Fab fragment.

159. The antibody of claim 152 which is selected from a chimeric antibody, a human antibody, or a humanized antibody.

160. The antibody of claim 152 which is produced in bacteria.

161. The antibody of claim 152 which is produced in CHO cells.

162. A method of determining the presence of a STEAP-1 protein in a sample suspected of containing said protein, said method comprising exposing said sample to an antibody of claim 152 and determining binding of said antibody to said STEAP-1 protein in said sample, wherein binding of the antibody to said protein is indicative of the presence of said protein in said sample.

163. The method of Claim 162 wherein said sample comprises a cell suspected of expressing said STEAP-1 protein.

164. The method of Claim 162 wherein said cell is B cell.

165. The method of Claim 162 wherein the antibody is covalently attached to a label selected from a fluorescent dye, a radioisotope, biotin, or a metal-complexing ligand.

166. A pharmaceutical formulation comprising the anti-STEAP-1 antibody of claim 152, and a pharmaceutically acceptable diluent, carrier or excipient.

167. The antibody of claim 152 wherein the antibody is covalently attached to an auristatin or a maytansinoid drug moiety whereby an antibody drug conjugate is formed.

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168. The antibody-drug conjugate of claim 167 comprising an antibody (Ab), and an auristatin or maytansinoid drug moiety (D) wherein the cysteine engineered antibody is attached through one or more free cysteine amino acids by a linker moiety (L) to D; the compound having Formula I:

$Ab–(L–D)_p$ I

where p is 1, 2, 3, or 4.

169. The antibody-drug conjugate compound of claim 167 wherein p is 2.

170. The antibody-drug conjugate compound of claim 167 wherein L has the formula:

$$—A_a—W_w—Y_y—$$

where:

A is a Stretcher unit covalently attached to a cysteine thiol of the cysteine engineered antibody (Ab);

a is 0 or 1;

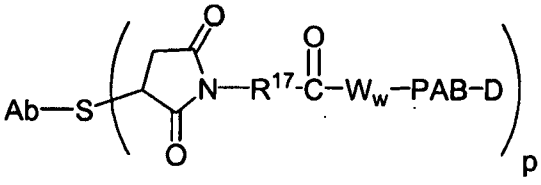
each W is independently an Amino Acid unit;

w is an integer ranging from 0 to 12;

Y is a Spacer unit covalently attached to the drug moiety; and

y is 0, 1 or 2.

171. The antibody-drug conjugate compound of claim 170 having the formula:



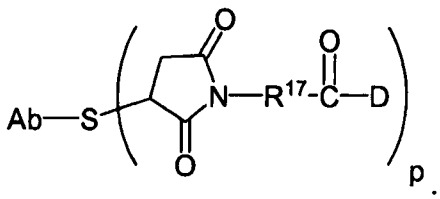
where PAB is para-aminobenzylcarbamoyl, and R^{17} is a divalent radical selected from $(CH_2)_r$, C_3-C_8 carbocyclyl, O–$(CH_2)_r$, arylene, $(CH_2)_r$–arylene, –arylene–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbocyclyl), (C_3-C_8 carbocyclyl)–$(CH_2)_r$, C_3-C_8 heterocyclyl, $(CH_2)_r$–(C_3-C_8 heterocyclyl), –(C_3-C_8 heterocyclyl)–$(CH_2)_r$–, –$(CH_2)_rC(O)NR^b(CH_2)_r$–, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, –$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$–, –$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$–$CH_2$–, –$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$–$CH_2$–, and –$(CH_2CH_2O)_rC(O)NR^b(CH_2)_r$– ; where R^b is H, C_1-C_6 alkyl, phenyl, or benzyl; and r is independently an integer ranging from 1 to 10.

172. The antibody-drug conjugate compound of claim 170 wherein W_w is valine-citrulline.

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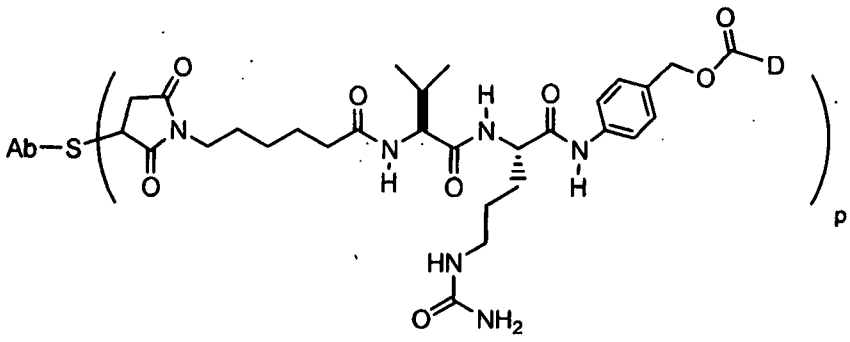
173. The antibody-drug conjugate compound of claim 170 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

174. The antibody-drug conjugate compound of claim 170 having the formula:



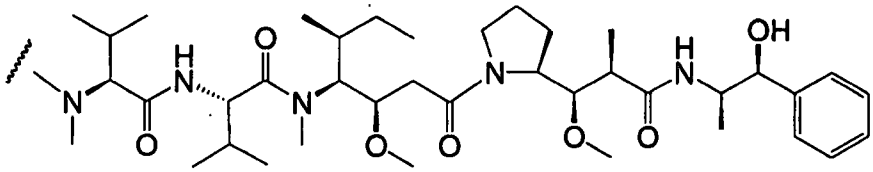
175. The antibody-drug conjugate compound of claim 174 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

176. The antibody-drug conjugate compound of claim 170 having the formula:



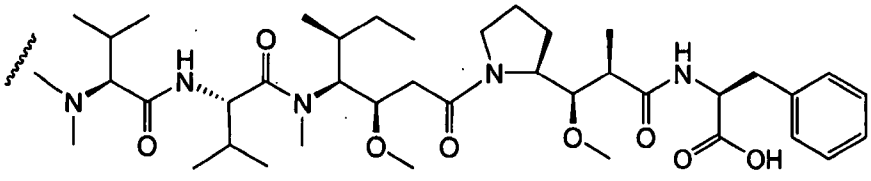
177. The antibody-drug conjugate compound of claim 168 wherein L is SMCC, SPP or BMPEO.

178. The antibody-drug conjugate compound of claim 168 wherein D is MMAE, having the structure:



wherein the wavy line indicates the attachment site to the linker L.

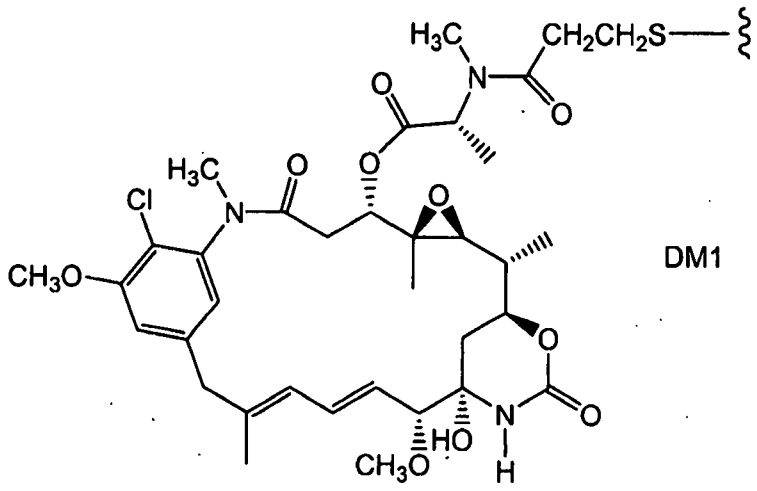
179. The antibody-drug conjugate compound of claim 168 wherein D is MMAF, having the structure:



wherein the wavy line indicates the attachment site to the linker L.

180. The antibody-drug conjugate compound of claim 168 wherein D is DM1, having the structure:

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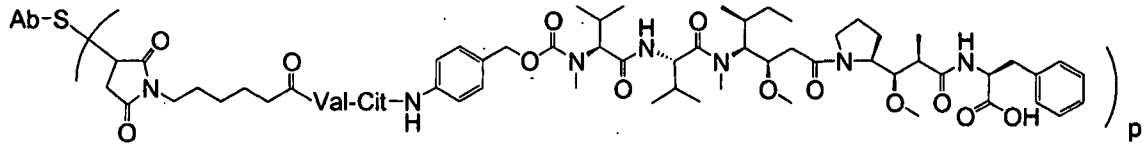
DM1

wherein the wavy line indicates the attachment site to the linker L.

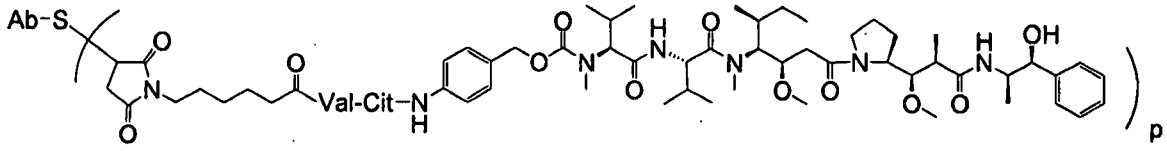
181. The antibody-drug conjugate compound of claim 167 wherein the parent anti-____ antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody, and an antibody fragment.

182. The antibody-drug conjugate compound of claim 167 wherein the antibody fragment is a Fab fragment.

183. An antibody-drug conjugate compound selected from the structures:

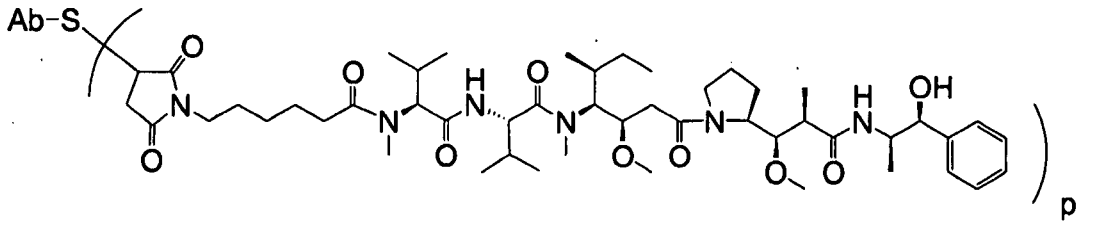


Ab-MC-vc-PAB-MMAF

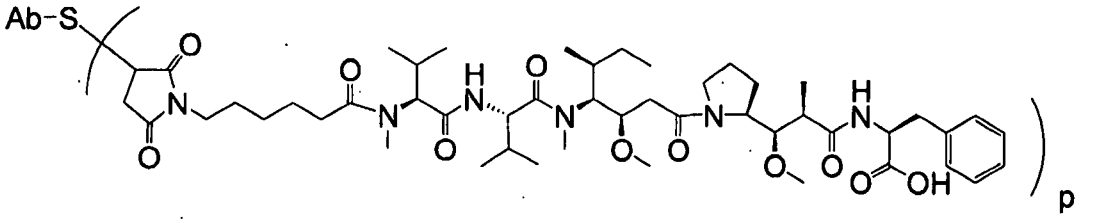


Ab-MC-vc-PAB-MMAE

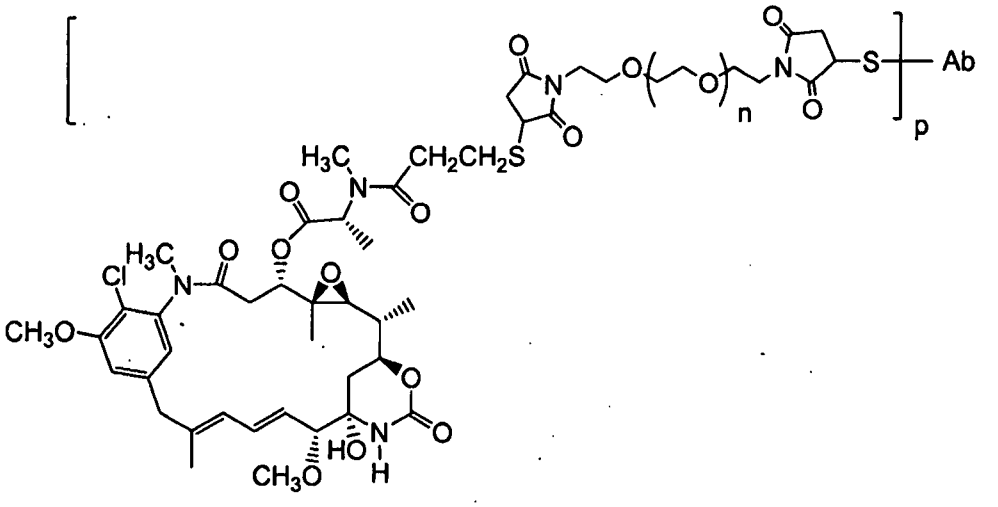
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Ab-MC-MMAE



Ab-MC-MMAF



Ab-BMPEO-DM1

wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or 4; and Ab is an anti-STEAP-1 antibody of claim 186.

184. The antibody drug conjugate of claim 167 wherein the auristatin is MMAE or MMAF.

185. The antibody drug conjugate of claim 168 wherein L is MC-val-cit-PAB or MC.

186. An assay for detecting B cells comprising:

(a) exposing cells to an antibody-drug conjugate compound of claim 165; and

(b) determining the extent of binding of the antibody-drug conjugate compound to the cells.

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187. A method of inhibiting cellular proliferation comprising treating mammalian cancerous B cells in a cell culture medium with an antibody-drug conjugate compound of claim 167, whereby proliferation of the cancerous B cells is inhibited.

188. A pharmaceutical formulation comprising the antibody drug conjugate of claim167, and a pharmaceutically acceptable diluent, carrier or excipient.

189. A method of treating cancer comprising administering to a patient the pharmaceutical formulation of claim 188.

190. The method of claim 189 wherein the cancer is selected from the group consisting of lymphoma, non-Hogkins lymphoma (NHL), aggressive NHL, relapsed aggressive NHL, relapsed indolent NHL, refractory NHL, refractory indolent NHL, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma, leukemia, hairy cell leukemia (HCL), acute lymphocytic leukemia (ALL), and mantle cell lymphoma.

191. The method of claim 189 wherein the patient is administered a cytotoxic agent in combination with the antibody-drug conjugate compound.

192. An article of manufacture comprising

the pharmaceutical formulation of claim186;

a container; and

a package insert or label indicating that the compound can be used to treat cancer characterized by the overexpression of a STEAP-1 polypeptide.

193. The article of manufacture of claim 192 wherein the cancer is selected from the group consisting of prostate cancer, lung cancer, colon cancer, bladder cancer, ovarian cance and Ewing's sarcoma.

194. A method for making an antibody drug conjugate compound comprising an anti-STEAP-1 antibody (Ab) of claim 152, and an auristatin or maytansinoid drug moiety (D) wherein the antibody is attached through the one or more engineered cysteine amino acids by a linker moiety (L) to D; the compound having Formula **I**:

$$Ab-(L-D)_p \qquad \mathbf{I}$$

where p is 1, 2, 3, or 4; the method comprising the steps of:

(a) reacting an engineered cysteine group of the antibody with a linker reagent to form antibody-linker intermediate Ab-L; and

(b) reacting Ab-L with an activated drug moiety D; whereby the antibody-drug conjugate is formed;

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or comprising the steps of:

(c) reacting a nucleophilic group of a drug moiety with a linker reagent to form drug-linker intermediate D-L; and

(d) reacting D-L with an engineered cysteine group of the antibody; whereby the antibody-drug conjugate is formed.

195. The method of claim 194 further comprising the step of expressing the antibody in chinese hamster ovary (CHO) cells.

196. The method of claim 195 further comprising the step of treating the expressed antibody with a reducing agent.

197. The method of claim 196 wherein the reducing agent is selected from TCEP and DTT.

198. The method of claim 196 further comprising the step of treating the expressed antibody with an oxidizing agent, after treating with the reducing agent.

199. The method of claim 198 wherein the oxidizing agent is selected from copper sulfate, dehydroascorbic acid, and air.

200. The antibody of claim 152 wherein the antibody comprises a heavy chain sequence selected from any one of SEQ ID NOS:9 or 10.

201. The antibody of claim 152 wherein the antibody comprises a light chain sequence of SEQ ID NO:6.

202. The antibody of claim 152 wherein the antibody comprises a light chain sequence of SEQ ID NO:6 and a heavy chain sequence of SEQ ID NO:9.

203. The antibody of claim 152 wherein the antibody comprises a light chain sequence of SEQ ID NO:6 and a heavy chain sequence of SEQ ID NO:10.

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ABSTRACT OF THE DISCLOSURE

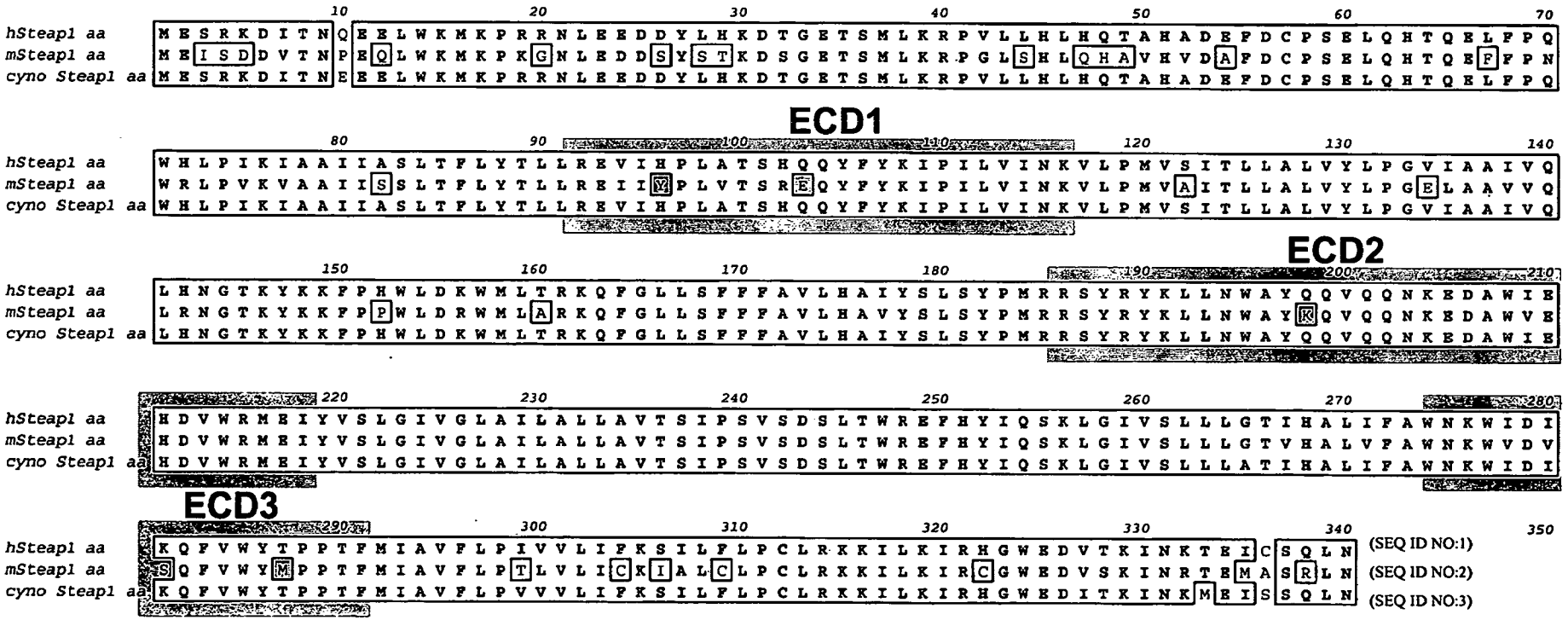
Anti-STEAP-1 antibodies and immunoconjugates thereof are provided. Methods of using anti-STEAP-1 antibodies and immunoconjugates thereof are provided.

5

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Figure 1



Alignment of Light Chain Sequences

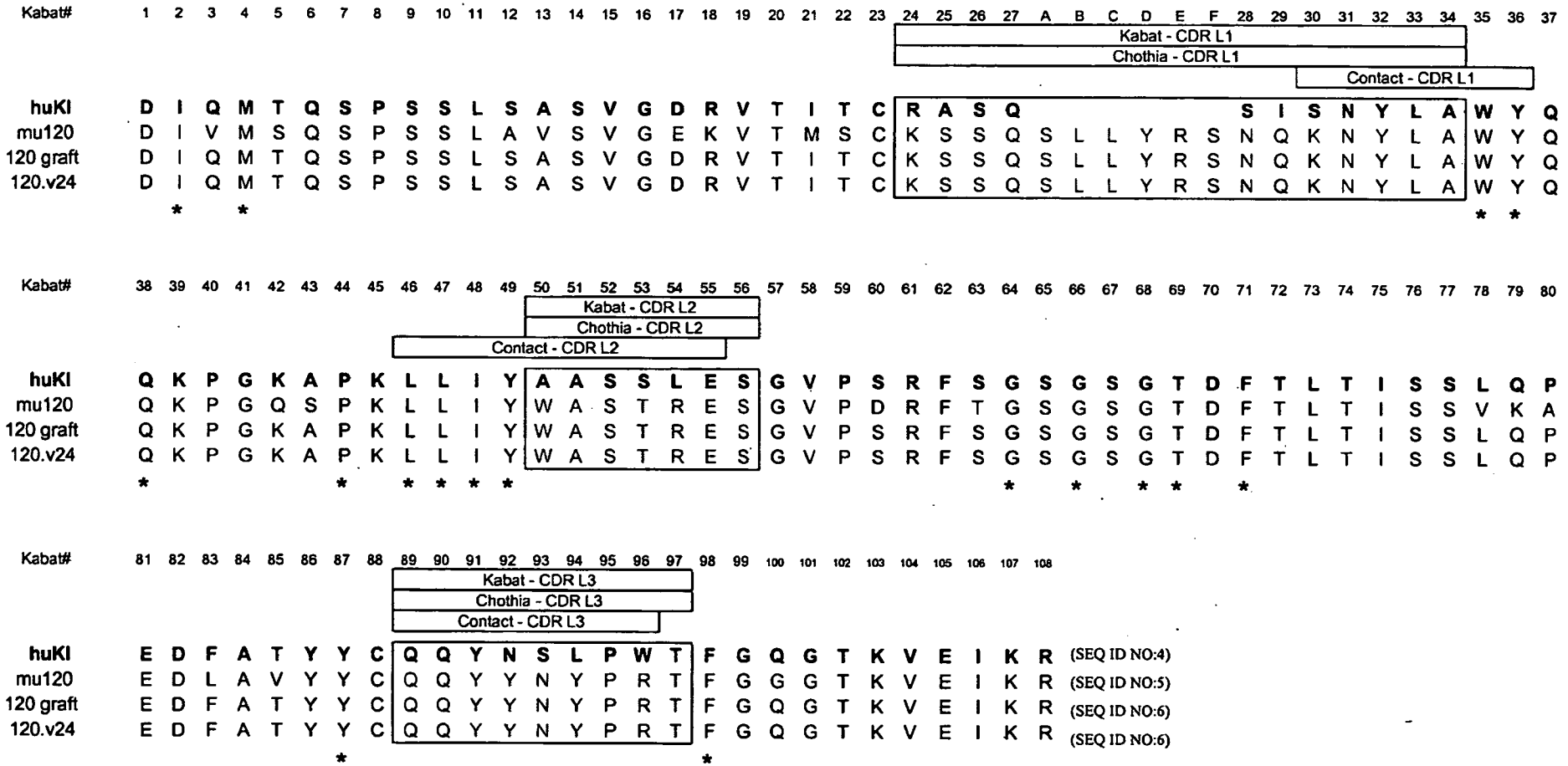


Figure 2A

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Alignment of Heavy Chain Sequences

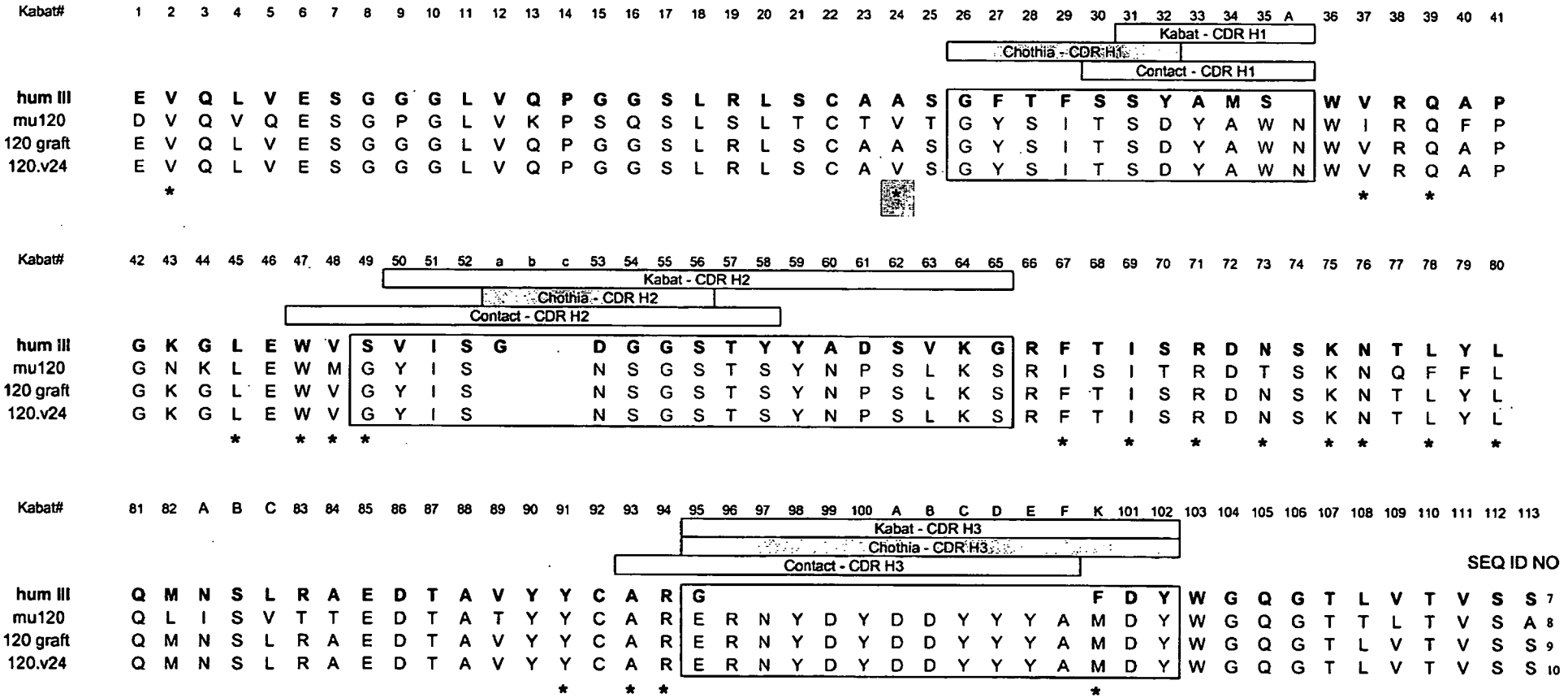


Figure 2B

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I

A	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T	-H1-	W V R Q A P G Q G L E W M G	-H2-
B	Q V Q L V Q S G A E V K K P G A S V K V S C K A S	-H1-	W V R Q A P G Q G L E W M	-H2-
C	Q V Q L V Q S G A E V K K P G A S V K V S C K A S	-H1-	W V R Q A P G Q G L E W M	-H2-
D	Q V Q L V Q S G A E V K K P G A S V K V S C K A S	-H1-	W V R Q A P G Q G L E W M	-H2-

II

A	Q V Q L Q E S G P G L V K P S Q T L S L T C T V S G G S V S	-H1-	W I R Q P P G K G L E W I G	-H2-
B	Q V Q L Q E S G P G L V K P S Q T L S L T C T V S	-H1-	W I R Q P P G K G L E W I	-H2-
C	Q V Q L Q E S G P G L V K P S Q T L S L T C T V S	-H1-	W I R Q P P G K G L E W I	-H2-
D	Q V Q L Q E S G P G L V K P S Q T L S L T C T V S	-H1-	W I R Q P P G K G L E W I	-H2-

III

A	E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S	-H1-	W V R Q A P G K G L E W V S	-H2-
B	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-
C	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-
D	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-

Acceptor - 1

A	E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K	-H1-	W V R Q A P G K G L E W V S	-H2-
B	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-
C	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-

Acceptor - 2

A	E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K	-H1-	W V R Q A P G K G L E W V S	-H2-
B	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-
C	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-
D	E V Q L V E S G G G L V Q P G G S L R L S C A A S	-H1-	W V R Q A P G K G L E W V	-H2-

F I G . 3A

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			SEQ ID NOs:
RVTITADTSTSTAYMELSSLRSEDTAVYYCAR	-H3-	WGQGTLVTVSS	26, 27, 28, 29
RVTITADTSTSTAYMELSSLRSEDTAVYYCAR	-H3-	WGQGTLVTVSS	30, 31, 28, 29
RVTITADTSTSTAYMELSSLRSEDTAVYYCA	-H3-	WGQGTLVTVSS	30, 31, 32, 29
RVTITADTSTSTAYMELSSLRSEDTAVYYC	-H3-	WGQGTLVTVSS	30, 31, 33, 29
RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	-H3-	WGQGTLVTVSS	34, 35, 36, 29
RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	-H3-	WGQGTLVTVSS	37, 38, 36, 29
RVTISVDTSKNQFSLKLSSVTAADTAVYYCA	-H3-	WGQGTLVTVSS	37, 38, 39, 29
RVTISVDTSKNQFSLKLSSVTAADTAVYYC	-H3-	WGQGTLVTVSS	37, 38, 40, 29
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	41, 42, 43, 29
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	44, 45, 43, 29
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA	-H3-	WGQGTLVTVSS	44, 45, 46, 29
RFTISRDNSKNTLYLQMNSLRAEDTAVYYC	-H3-	WGQGTLVTVSS	44, 45, 47, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSR	-H3-	WGQGTLVTVSS	48, 42, 49, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCSR	-H3-	WGQGTLVTVSS	44, 45, 49, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCS	-H3-	WGQGTLVTVSS	44, 45, 50, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	48, 42, 51, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	44, 45, 51, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYCA	-H3-	WGQGTLVTVSS	44, 45, 52, 29
RFTISADTSKNTAYLQMNSLRAEDTAVYYC	-H3-	WGQGTLVTVSS	44, 45, 53, 29

FIG. 3B

κv1 DIQMTQSPSSLSASVGDRVTITC -L1- WYQQKPGKAPKLLIY -L2- GVPSRFSGSGSGTDFTLTISSLQP

κv1 DIQMTQSPSSLSASVGDRVTITC -L1- WYQQKPGKAPKLLI -L2- GVPSRFSGSGSGTDFTLTISSLQP

κv2 DIVMTQSPLSLPVTPGEPASISC -L1- WYLQKPGQSPQLLIY -L2- GVPDRFSGSGSGTDFTLKISRVEA

κv3 EIVLTQSPGTLSLSPGERATLSC -L1- WYQQKPGQAPRLLIY -L2- GIPDRFSGSGSGTDFTLTISRLEP

κv4 DIVMTQSPDSLAVSLGERATINC -L1- WYQQKPGQPPKLLIY -L2- GVPDRFSGSGSGTDFTLTISSLQA

FIG. 4A

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E D F A T Y Y C	-L3-	F G Q G T K V E I K	SEQ ID NOs:54, 55, 56, 57
E D F A T Y Y C	-L3-	F G Q G T K V E I K	SEQ ID NOs:54, 58, 56, 57
E D V G V Y Y C	-L3-	F G Q G T K V E I K	SEQ ID NOs:58, 59, 60, 57
E D F A V Y Y C	-L3-	F G Q G T K V E I K	SEQ ID NOs:61, 62, 63, 57
E D V A V Y Y C	-L3-	F G Q G T K V E I K	SEQ ID NOs:64, 65, 66, 57

FIG. 4B

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```
          230        240        250        260        270
humIgG1     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV
humIgG2     PAP-PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV
humIgG3     PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYV
humIgG4     PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
               ****                                    *     * *

          280        290        300        310        320
humIgG1     DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG2     DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
humIgG3     DGVEVHNAKTKPREEQFNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALP
humIgG4     DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP
                           *    *         *                   *

          330        340        350        360        370
humIgG1     APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
                                          D L
humIgG2     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG3     APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
humIgG4     SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
            **       *                  *

          380        390        400        410        420
humIgG1     EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG2     EWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
humIgG3     EWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
humIgG4     EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMH
               *        *    *              *          *   *

          430        440
humIgG1     EALHNHYTQKSLSLSPGK  (SEQ ID NO:85)
humIgG2     EALHNHYTQKSLSLSPGK  (SEQ ID NO:86)
humIgG3     EALHNRFTQKSLSLSPGK  (SEQ ID NO:87)
humIgG4     EALHNHYTQKSLSLSLGK  (SEQ ID NO:88)
               **        *
```

A Kappa light chain constant region consensus sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:89)

Figure 5

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Fig. 6A

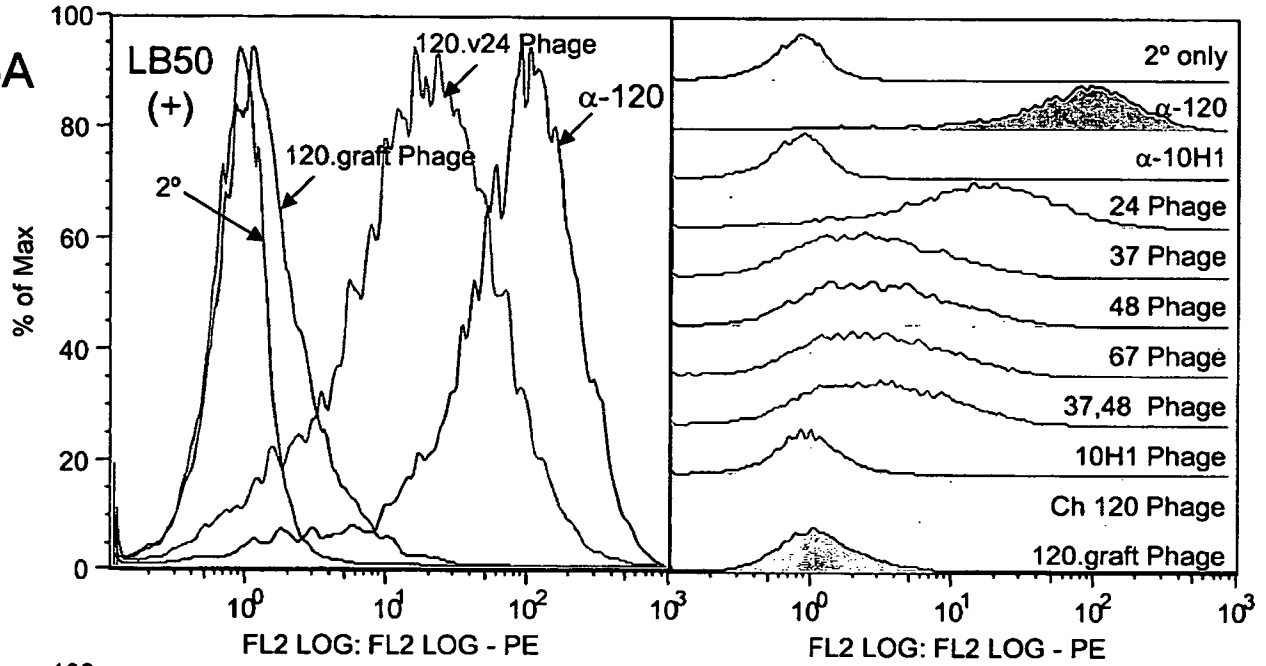


Fig. 6C

Fig. 6B

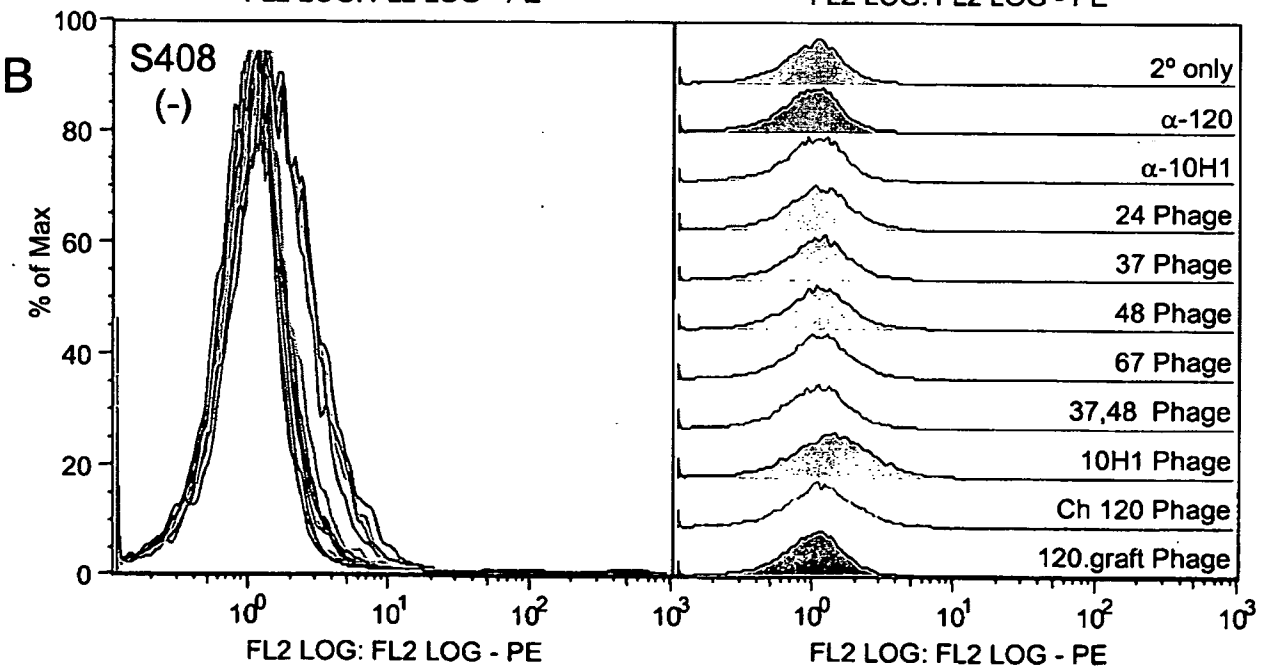


Fig. 6D

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FACs:

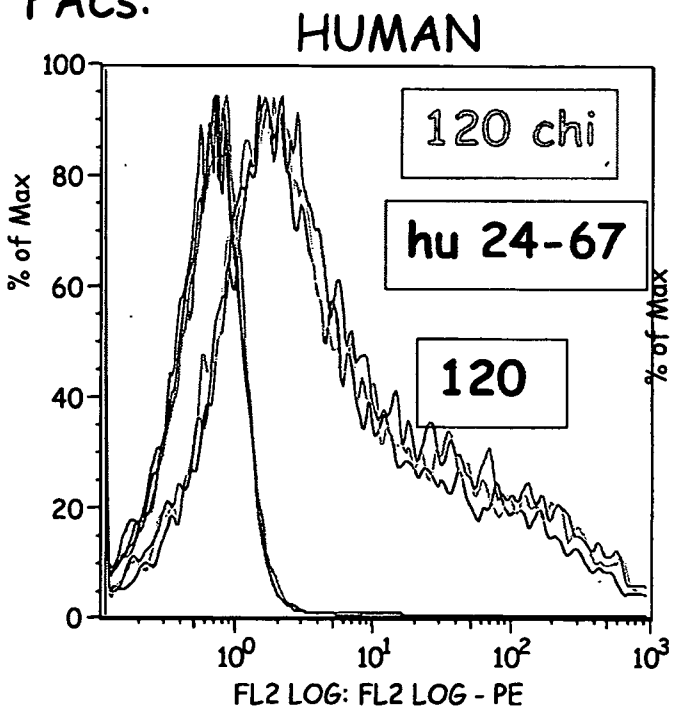


Fig. 7A

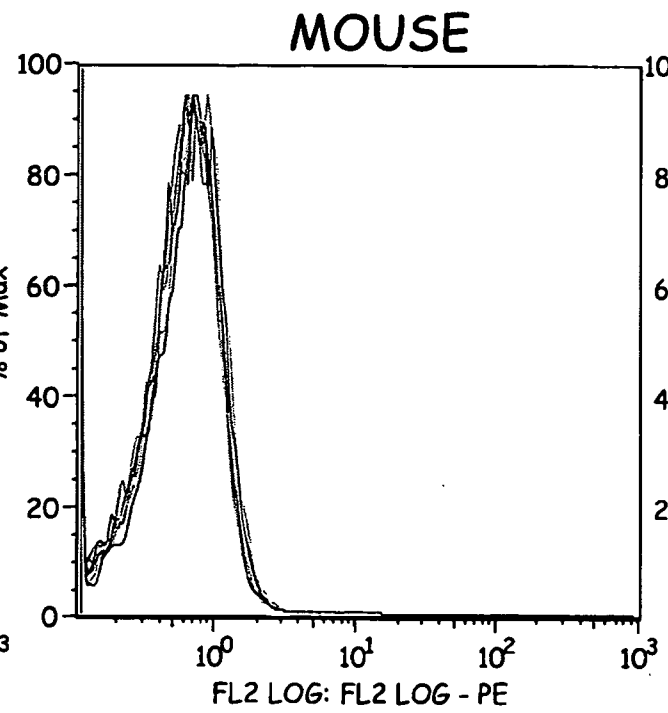


Fig. 7B

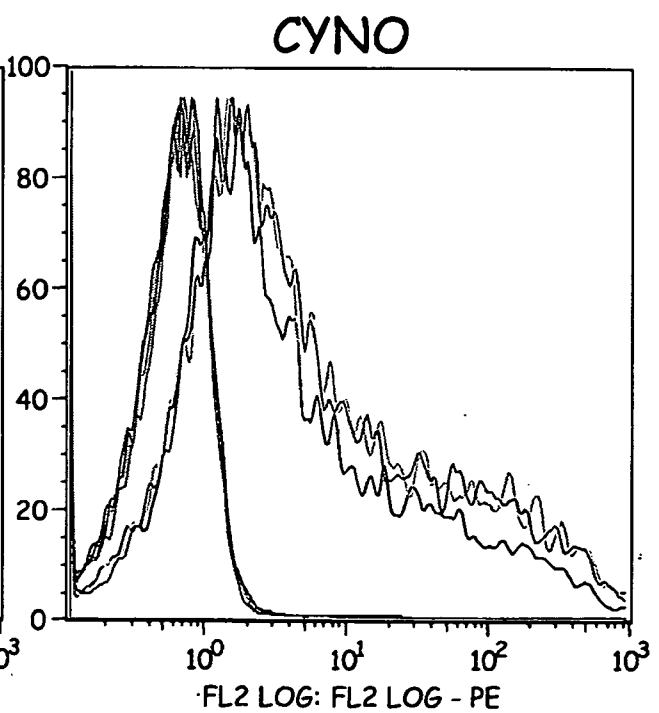


Fig. 7C

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FACs:

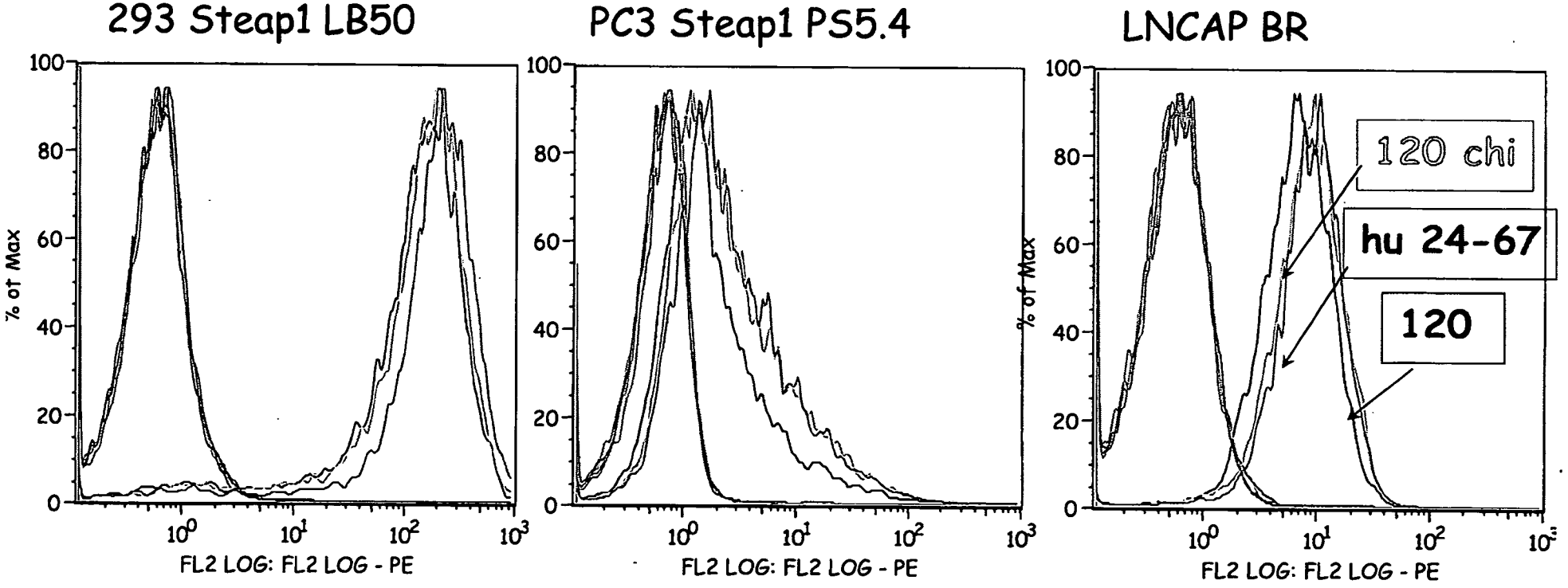
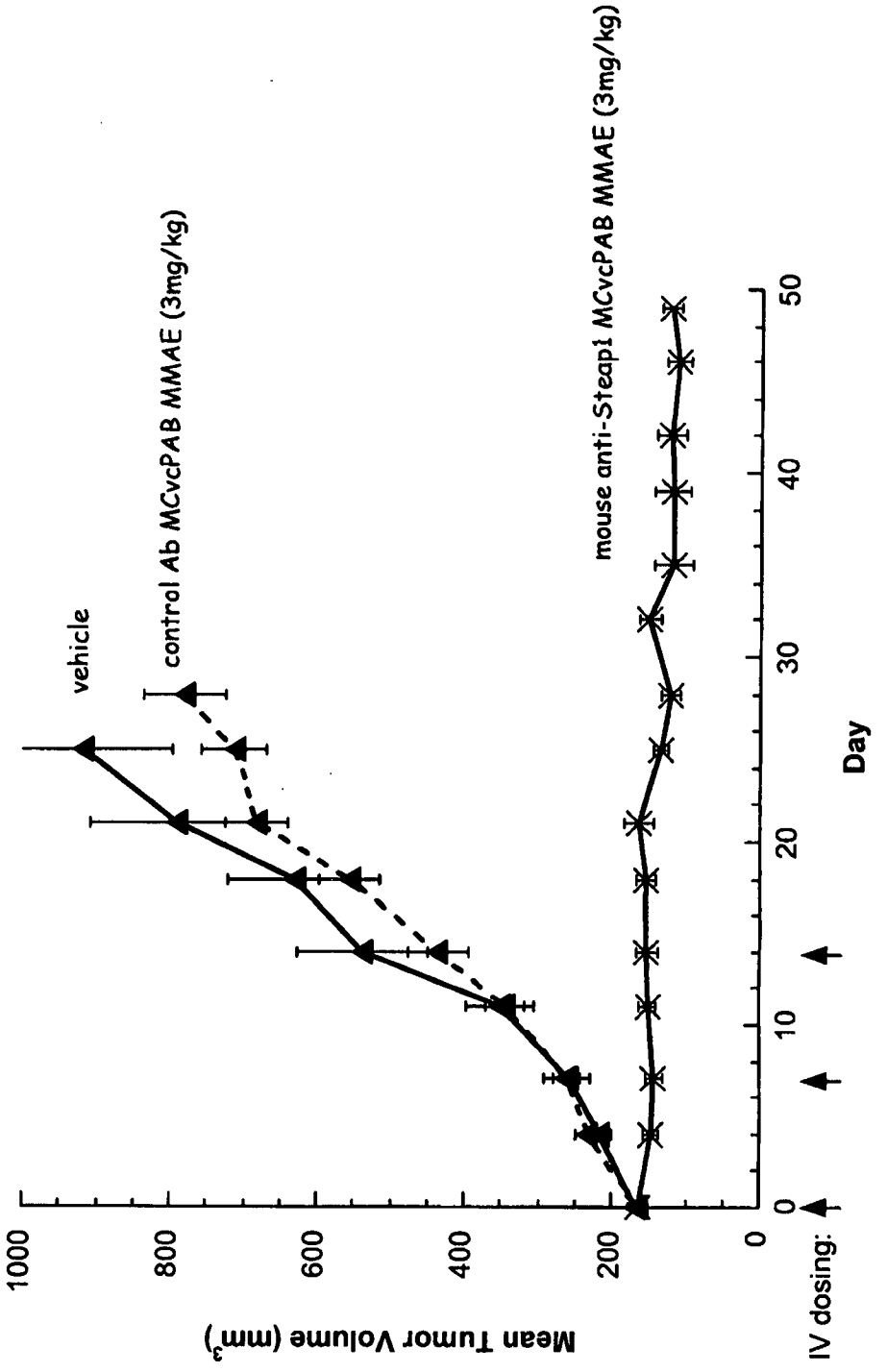


Fig. 7D

Fig. 7E

Fig. 7F

Figure 8A



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Figure 8B

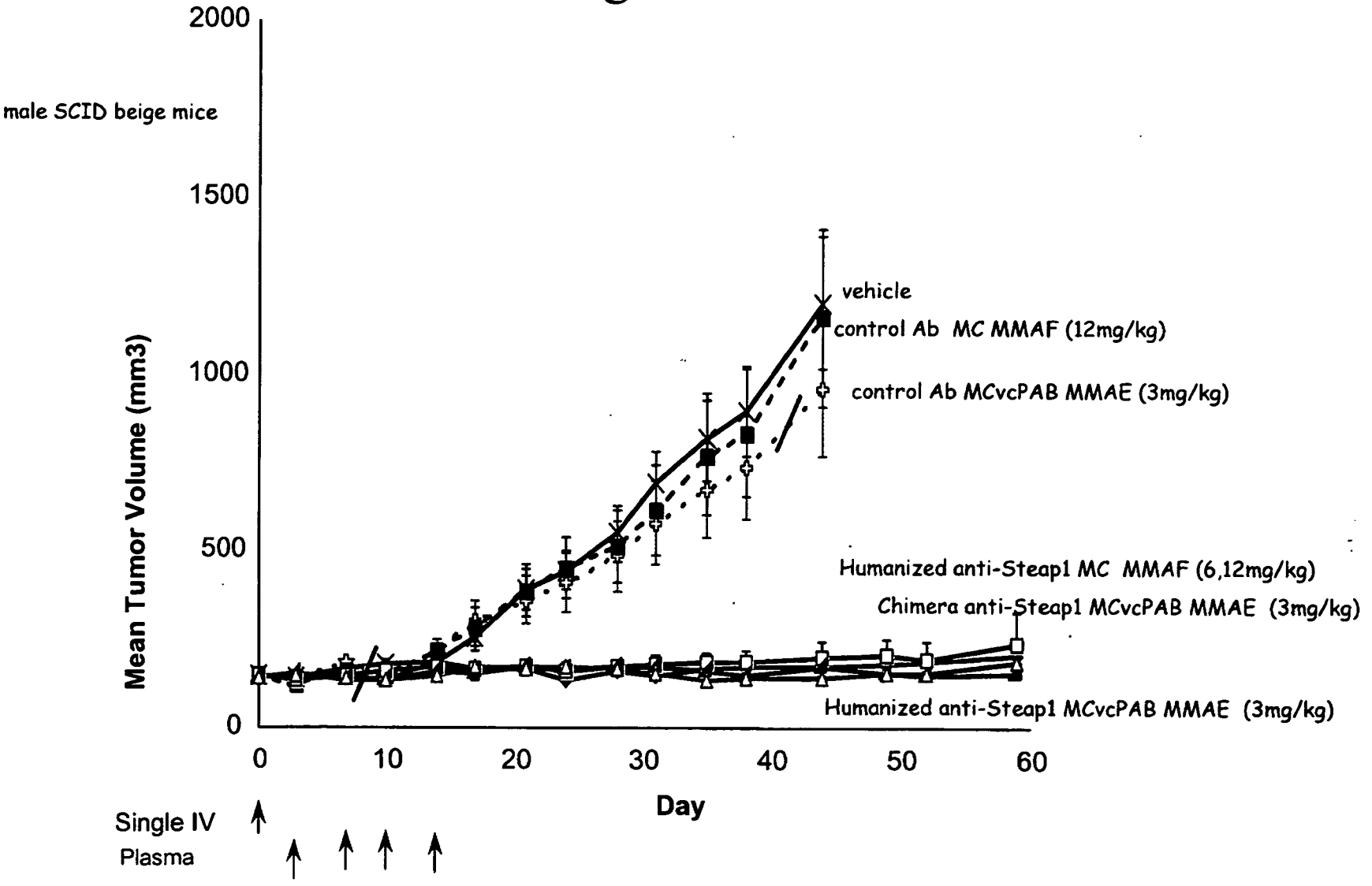
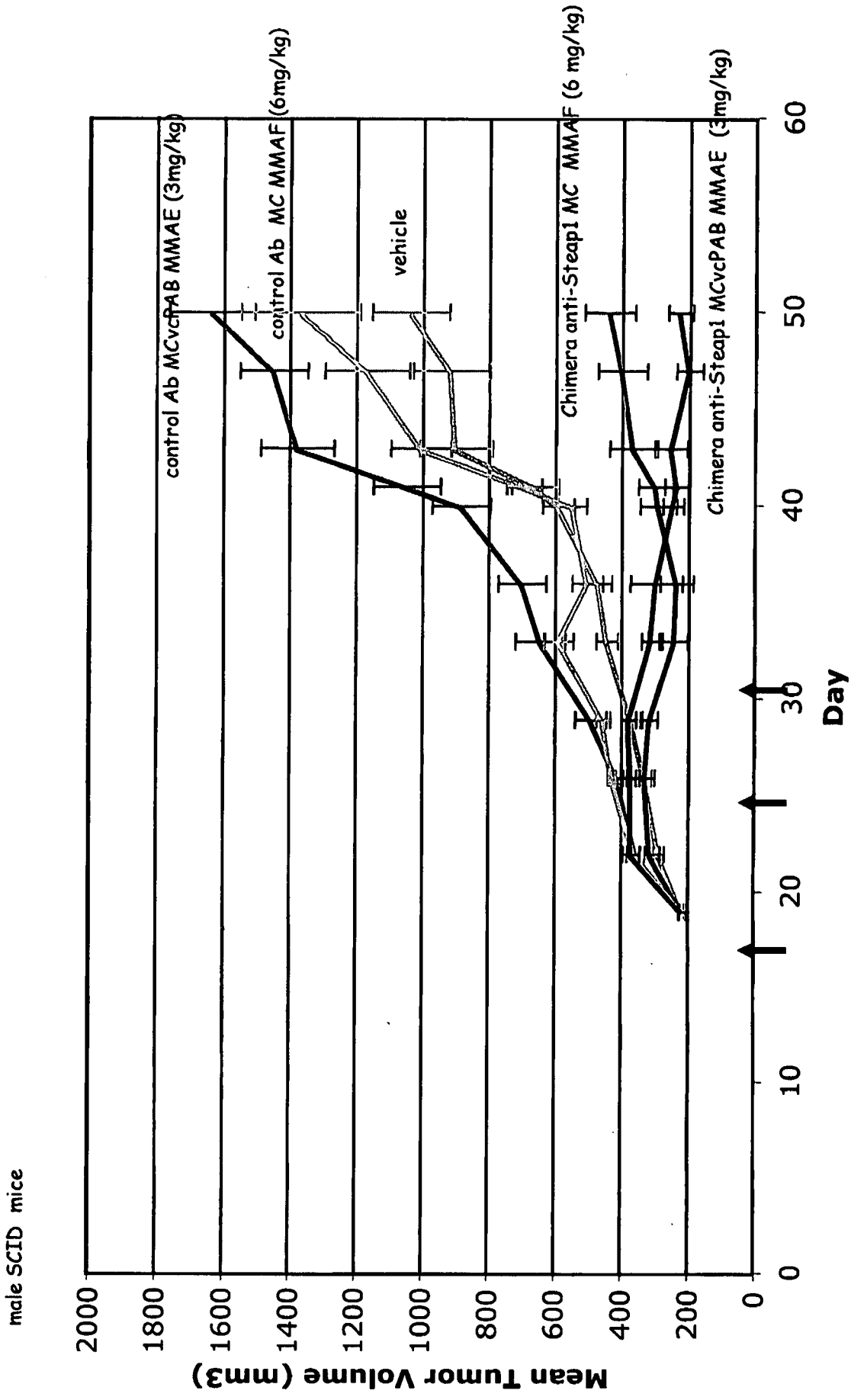
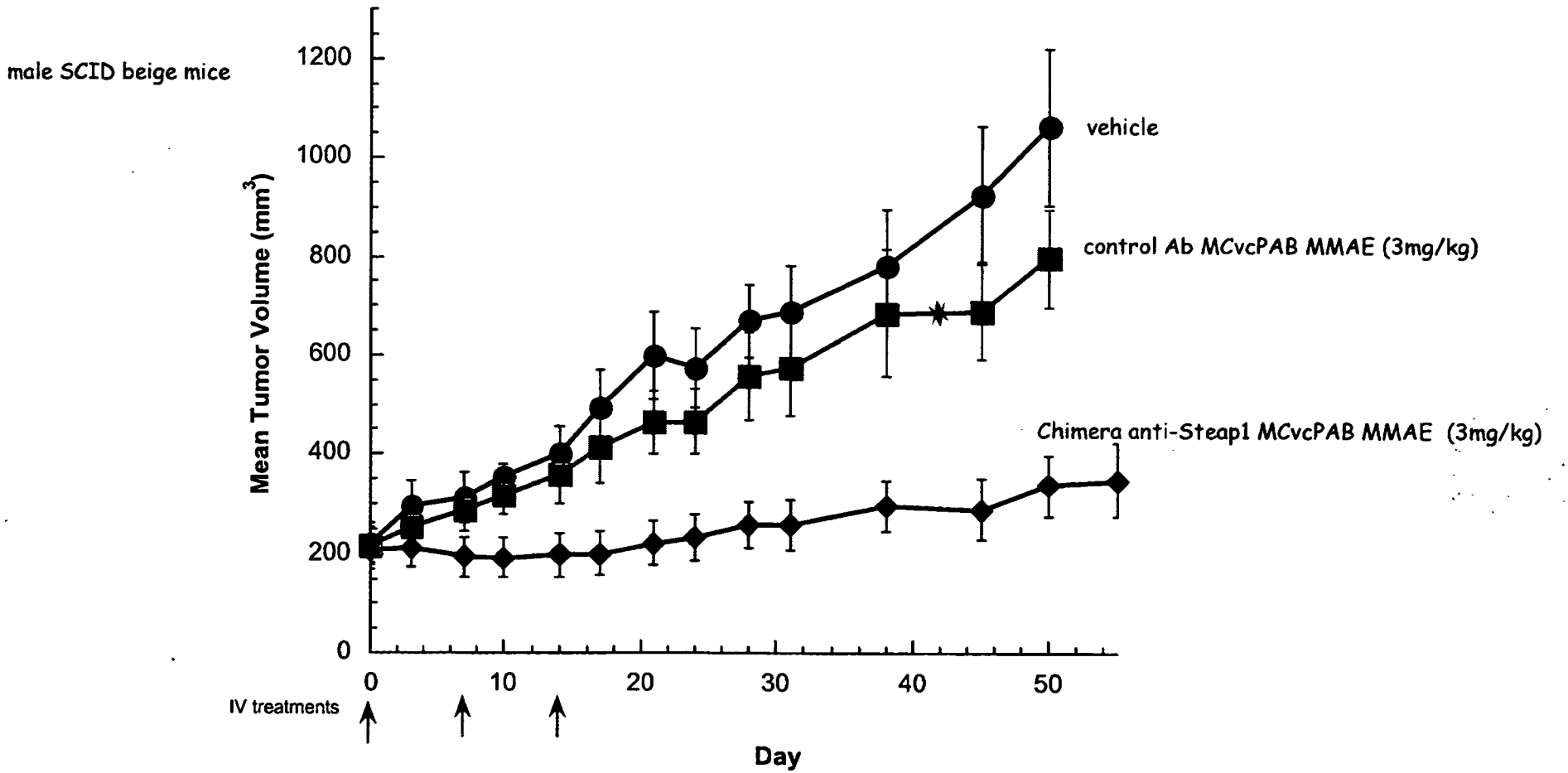


Figure 9



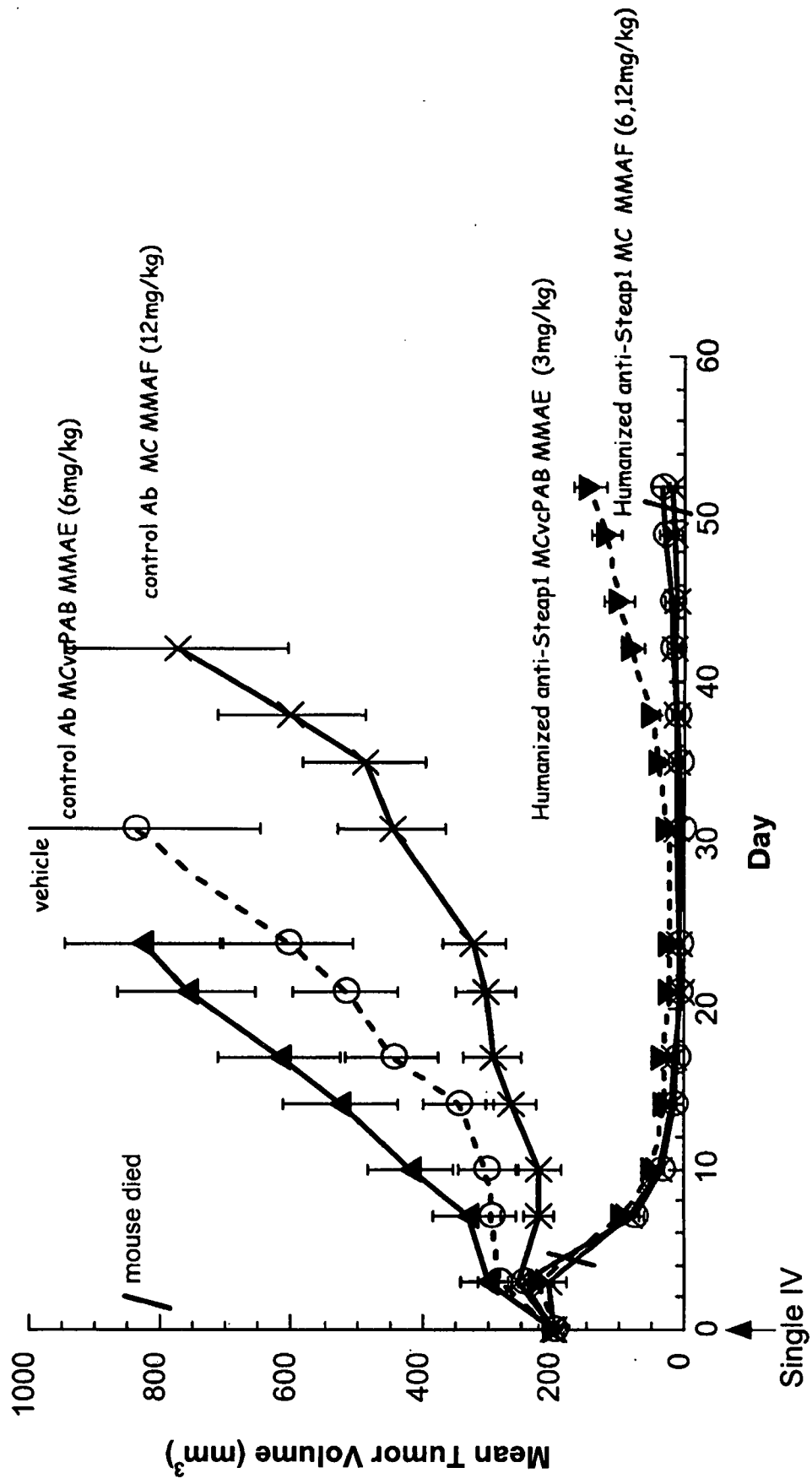
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Figure 10



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Figure 11



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Schematic Diagram of STEAP-1 in the Cell Membrane

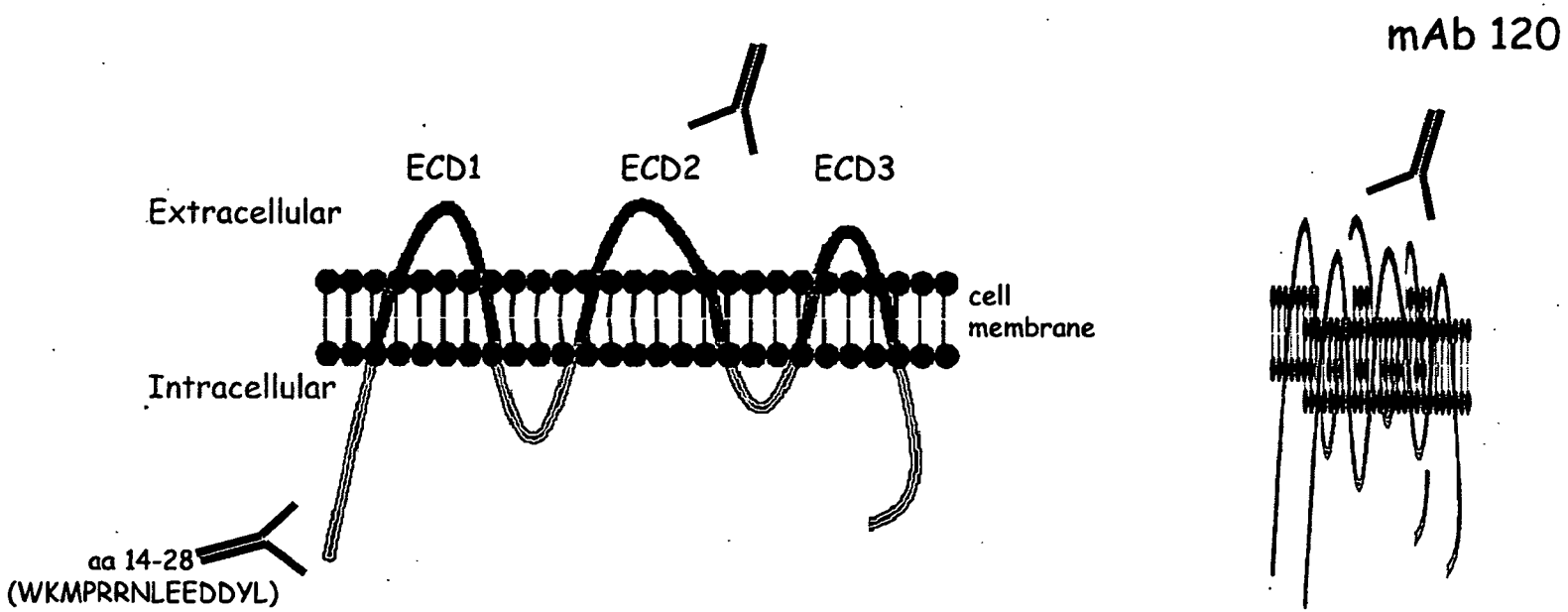


Figure 12

Expression of STEAP1 in prostate cancer models

Exogenous models *Endogenous models*

293 STEAP1 LB50 PC3 STEAP1 PS5.4 LNCaP LuCAP 77

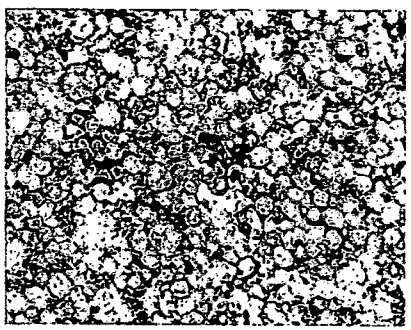


Fig. 13A

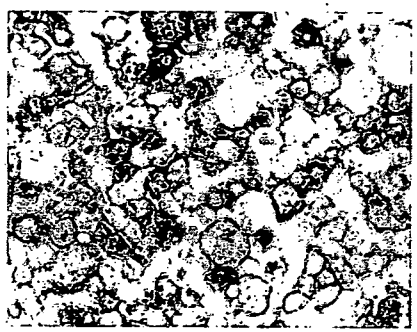


Fig. 13B

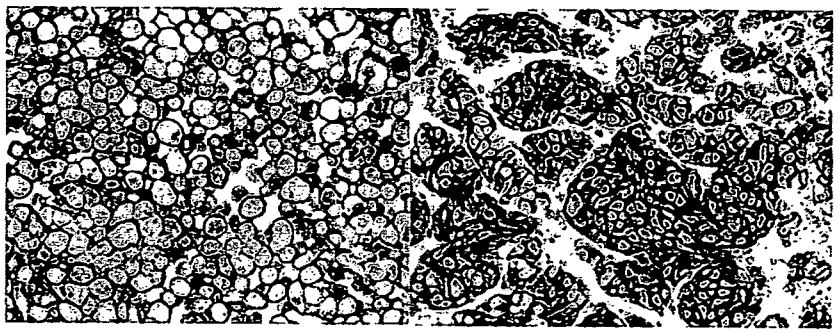


Fig. 13C

Fig. 13D

Figures 13A-13D

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Fig. 14A

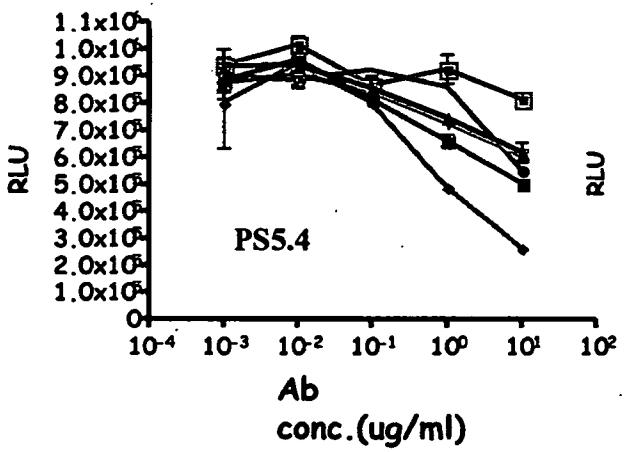


Fig. 14B

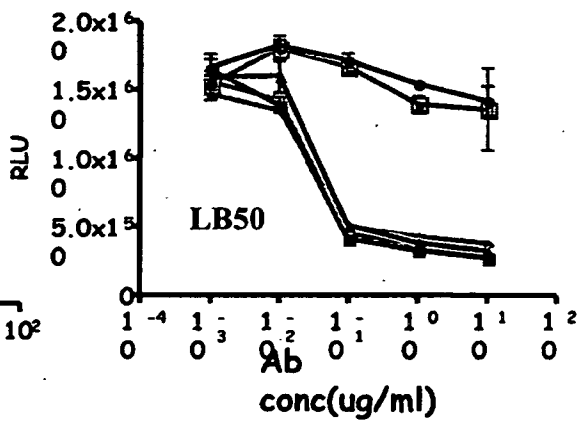


Fig. 14C

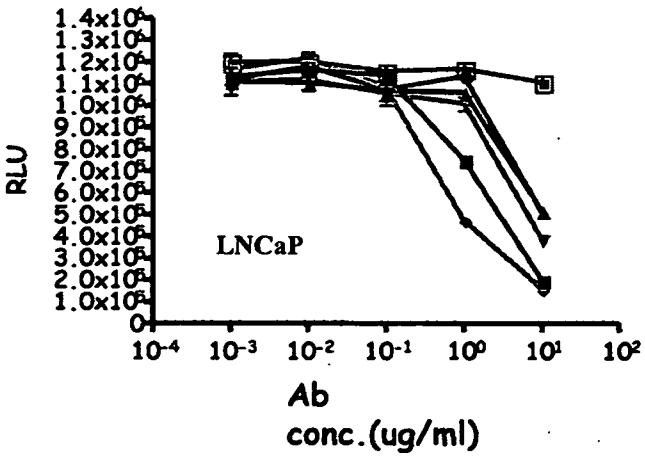


Fig. 14D

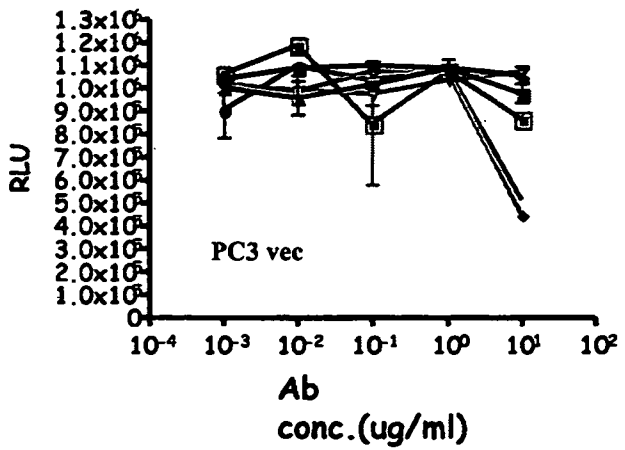
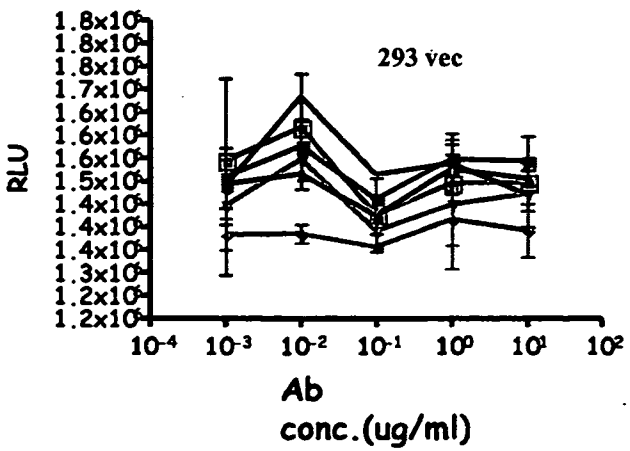
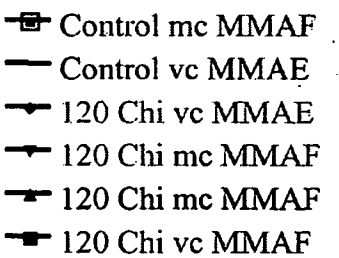


Fig. 14 E





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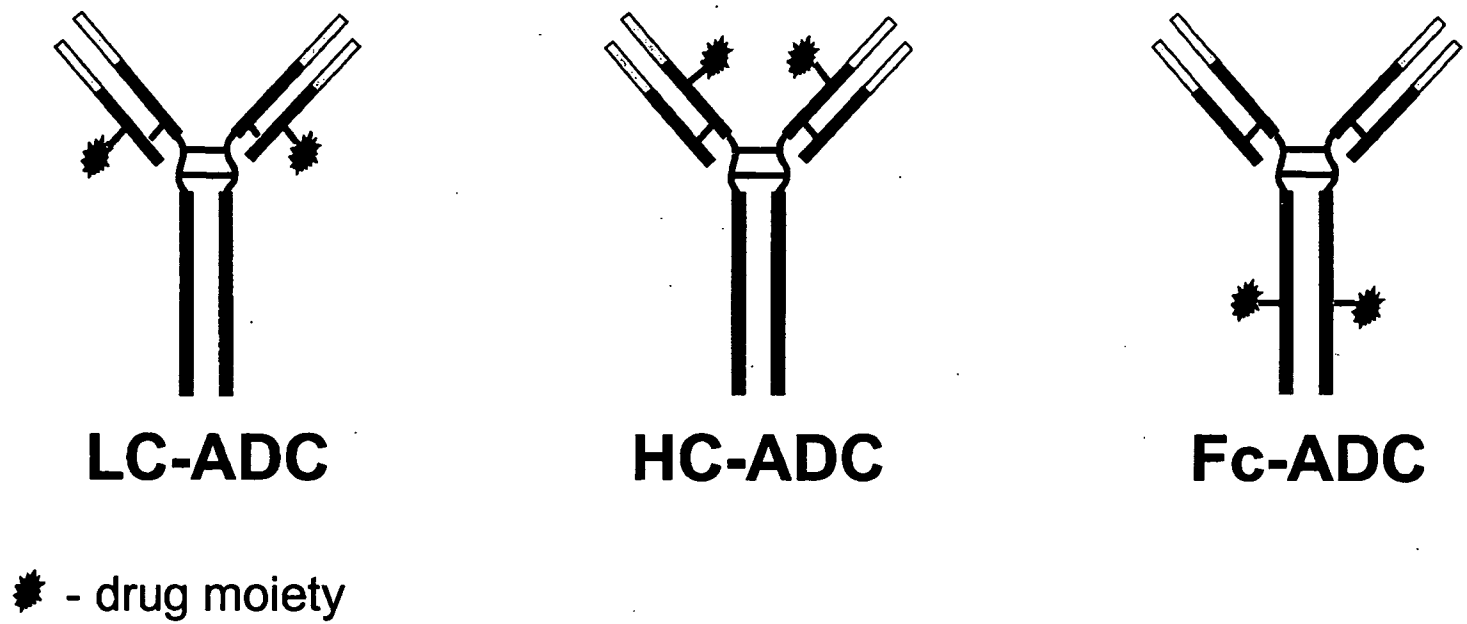


Figure 15

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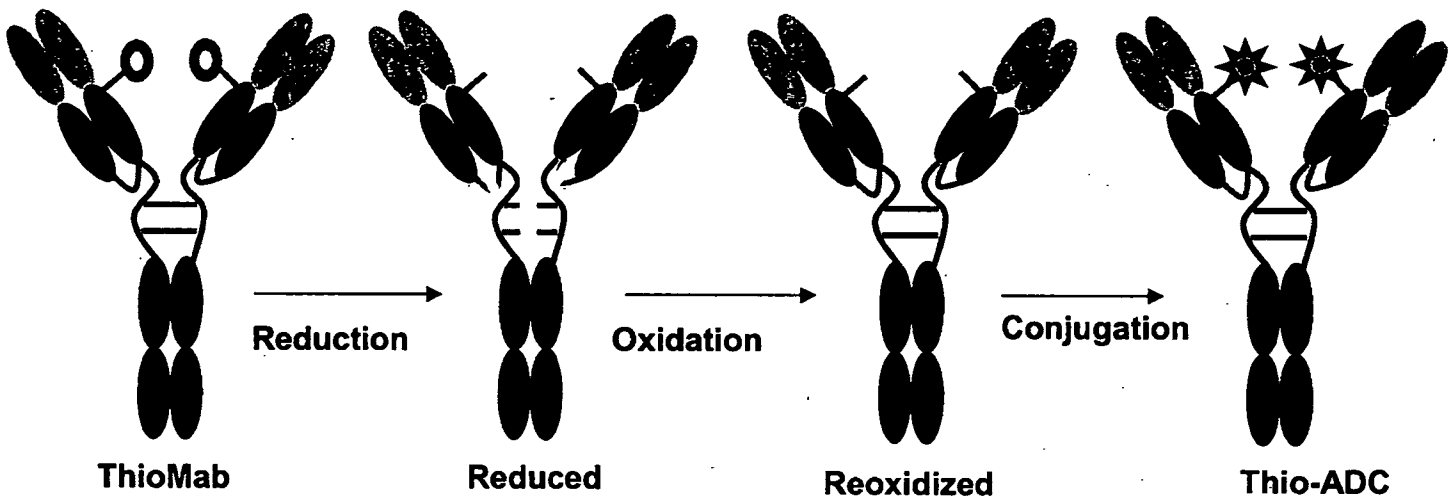


Figure 16

Thio-LC Variant (V205C) with corresponding sequential numbering

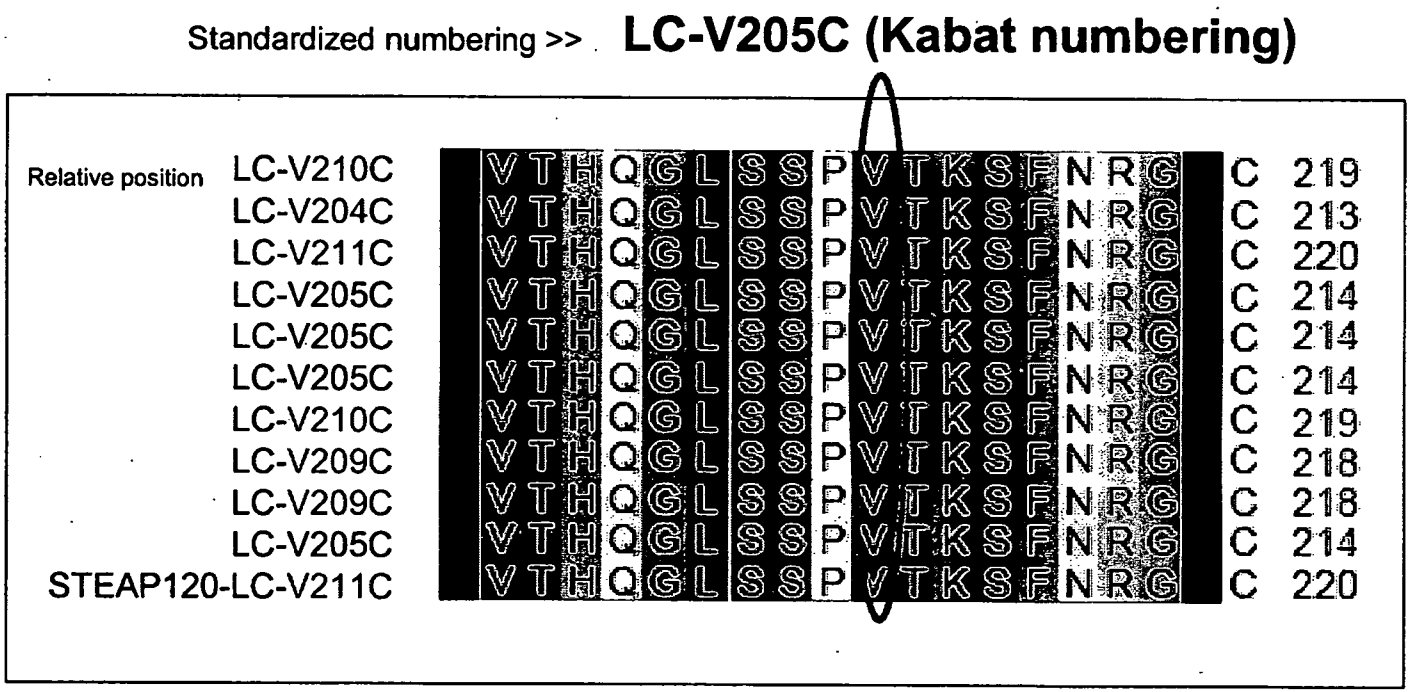


Figure 17A

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Thio-HC Variant (A121C) with corresponding sequential numbering

Standardized numbering >> **HC-A118C (EU Numbering)**

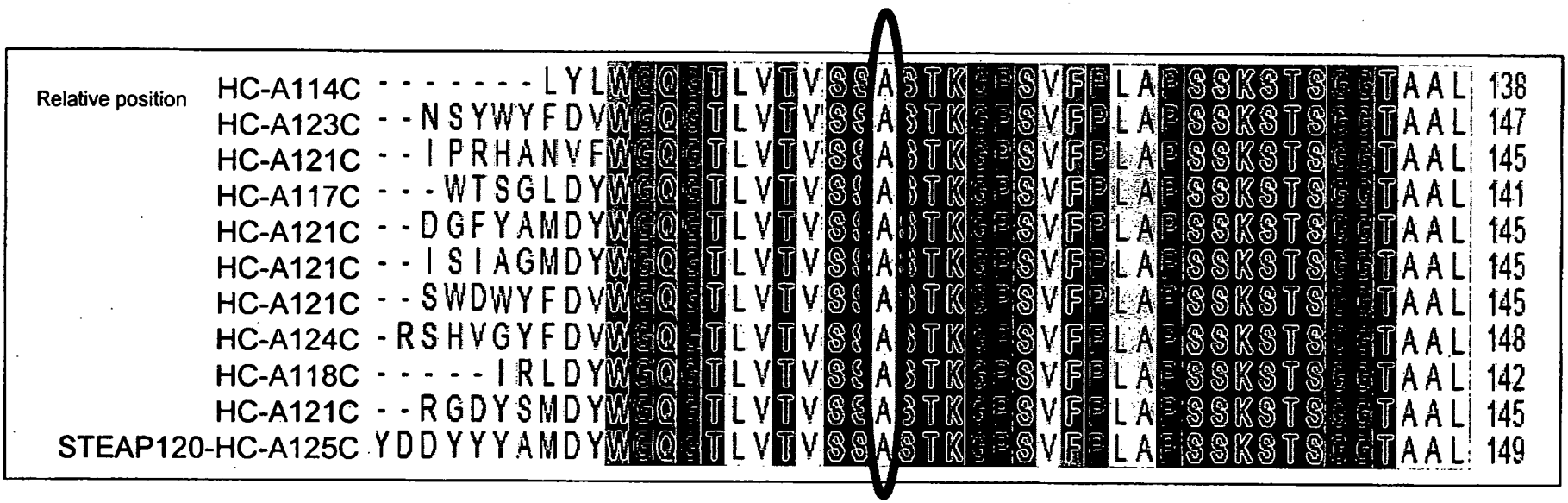


Figure 17B

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Thio-Fc Variant (S400C) with corresponding sequential numbering

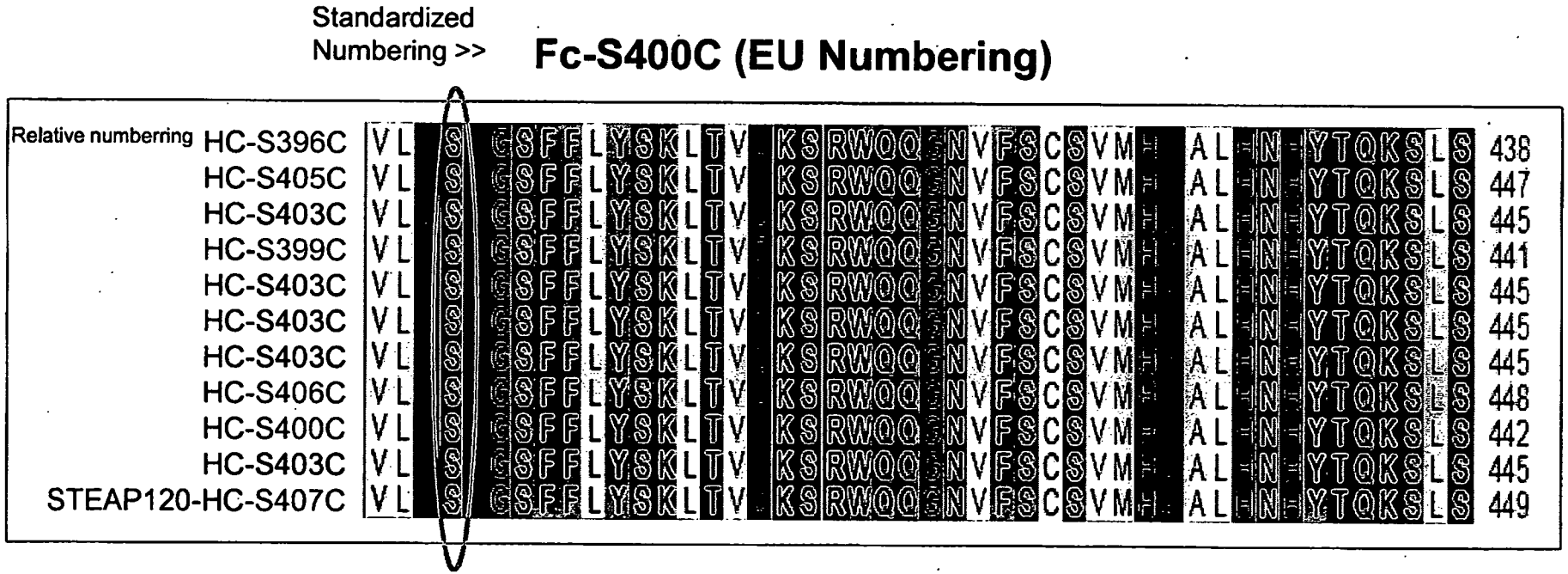


Figure 17C

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Fig. 18A

293 Steap1 c.LB50

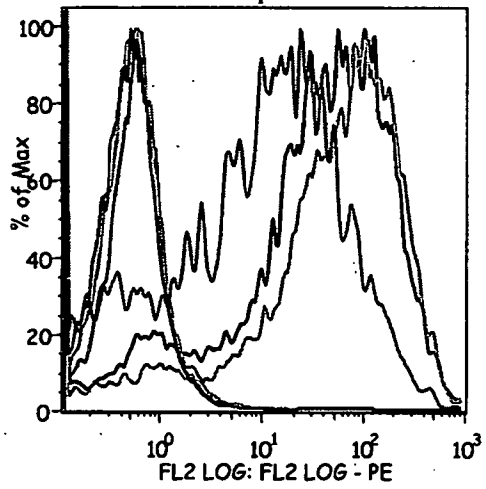


Fig. 18B

PC3 Steap1 c.PS5.4

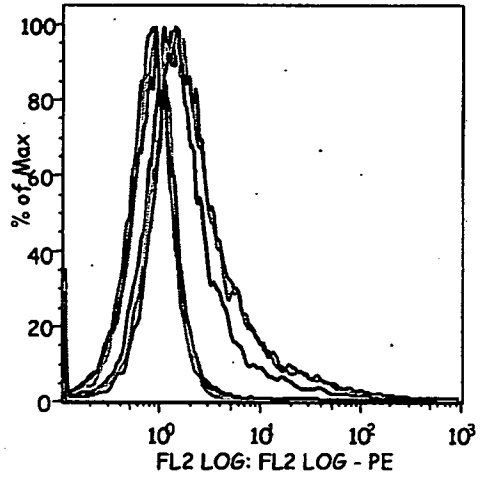
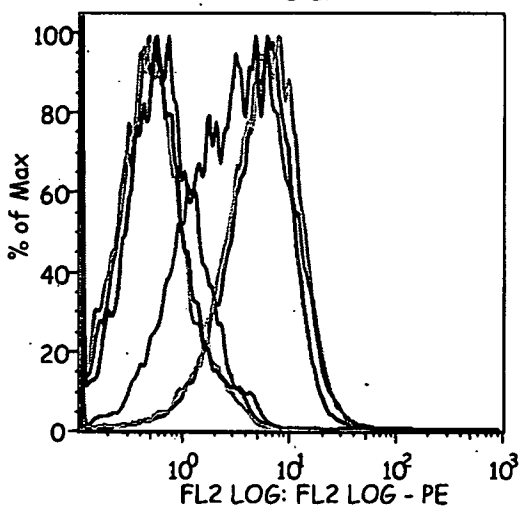


Fig. 18C

LNCaP



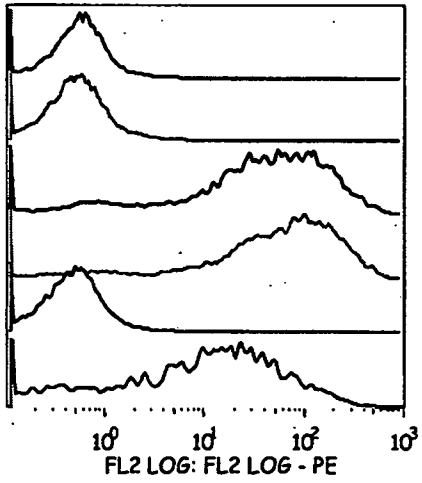


Fig. 18D

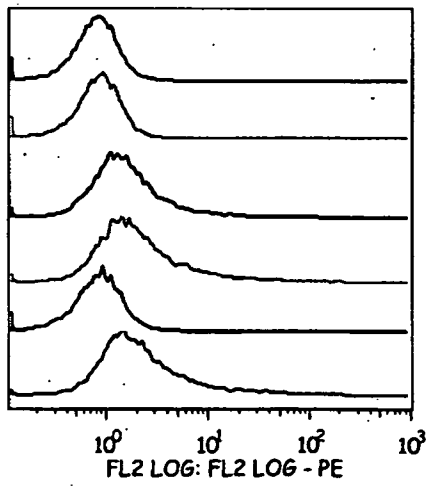


Fig. 18E

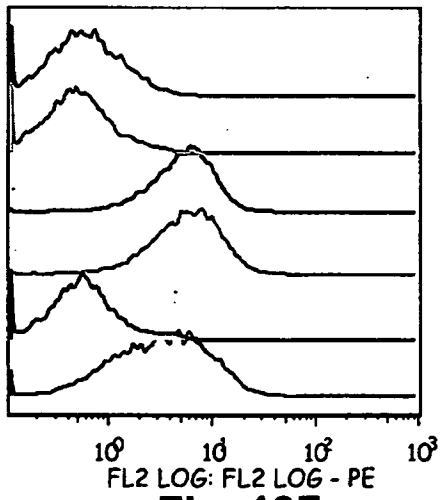


Fig. 18F

2nd only

TDC control (vcE)

huSteap1 TDC (LC V211C) vcE

huSteap1 TDC (HCA125C) vcE

ADC std control (vcE)

huSteap1 ADC (std) vcE

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Fig. 19A

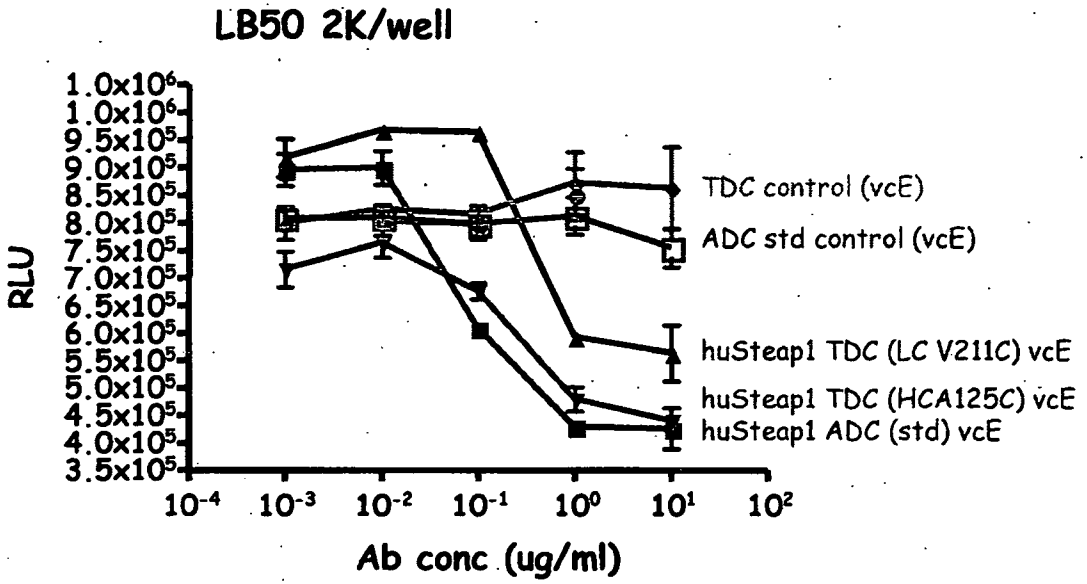
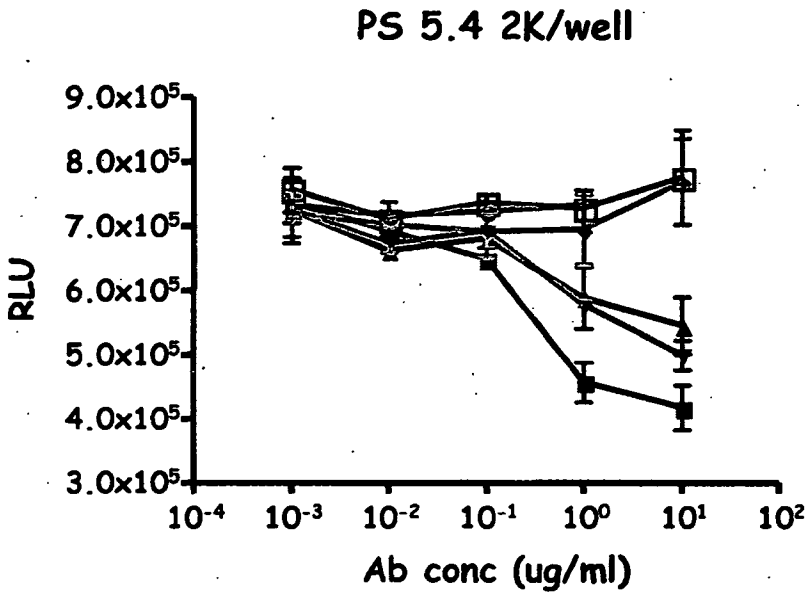


Fig. 19B



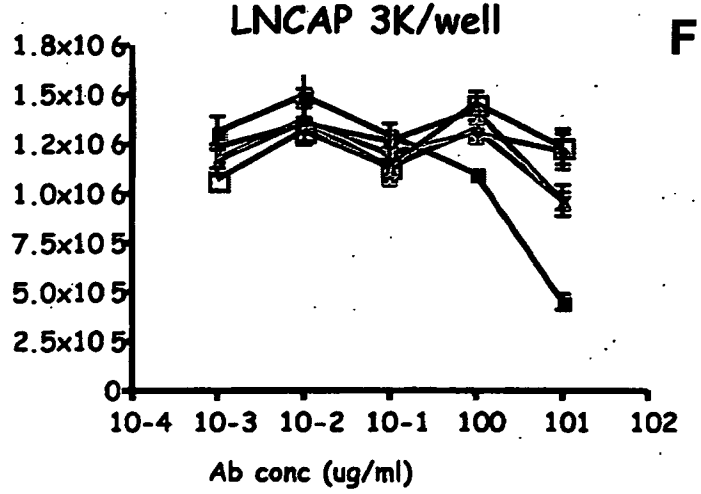
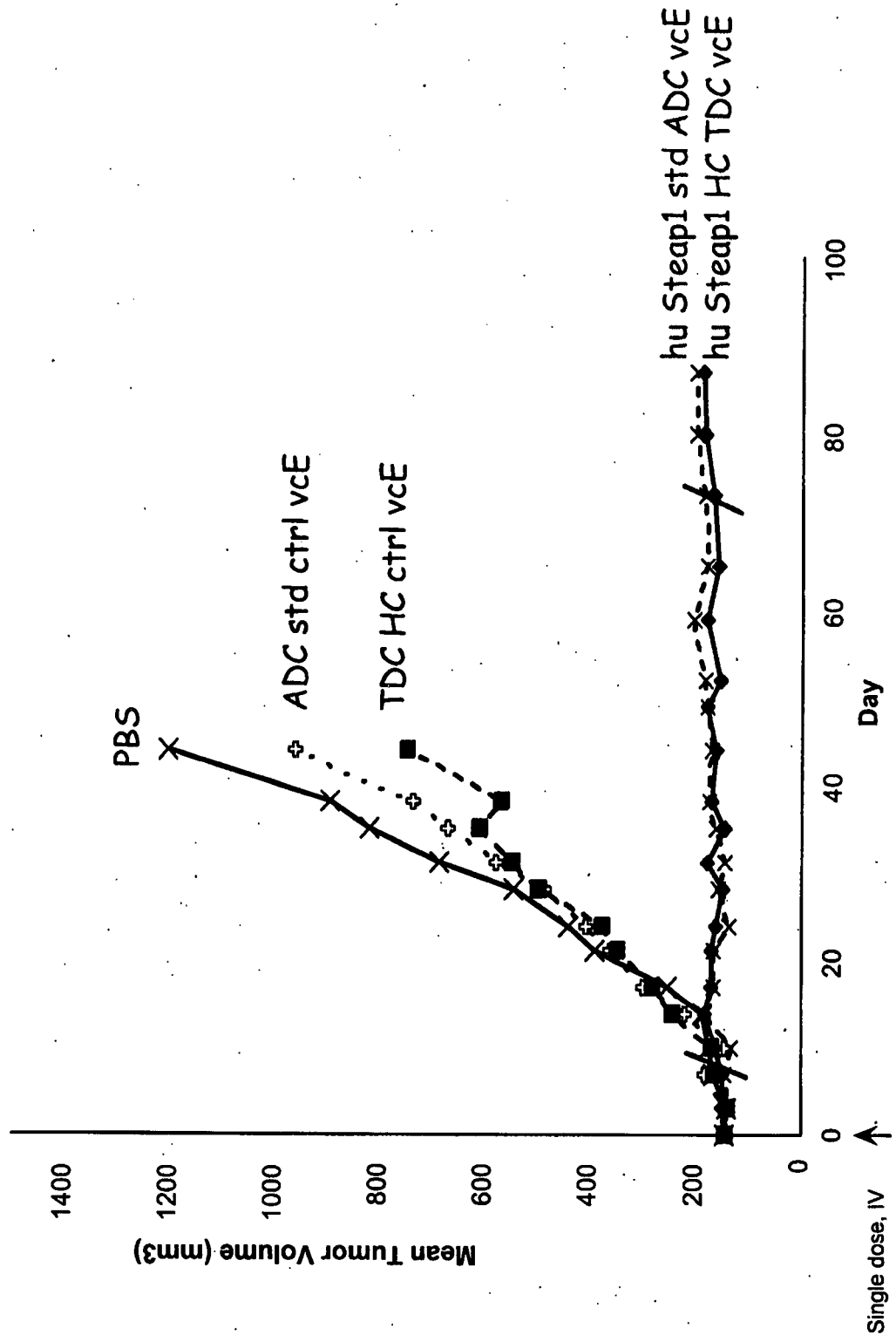


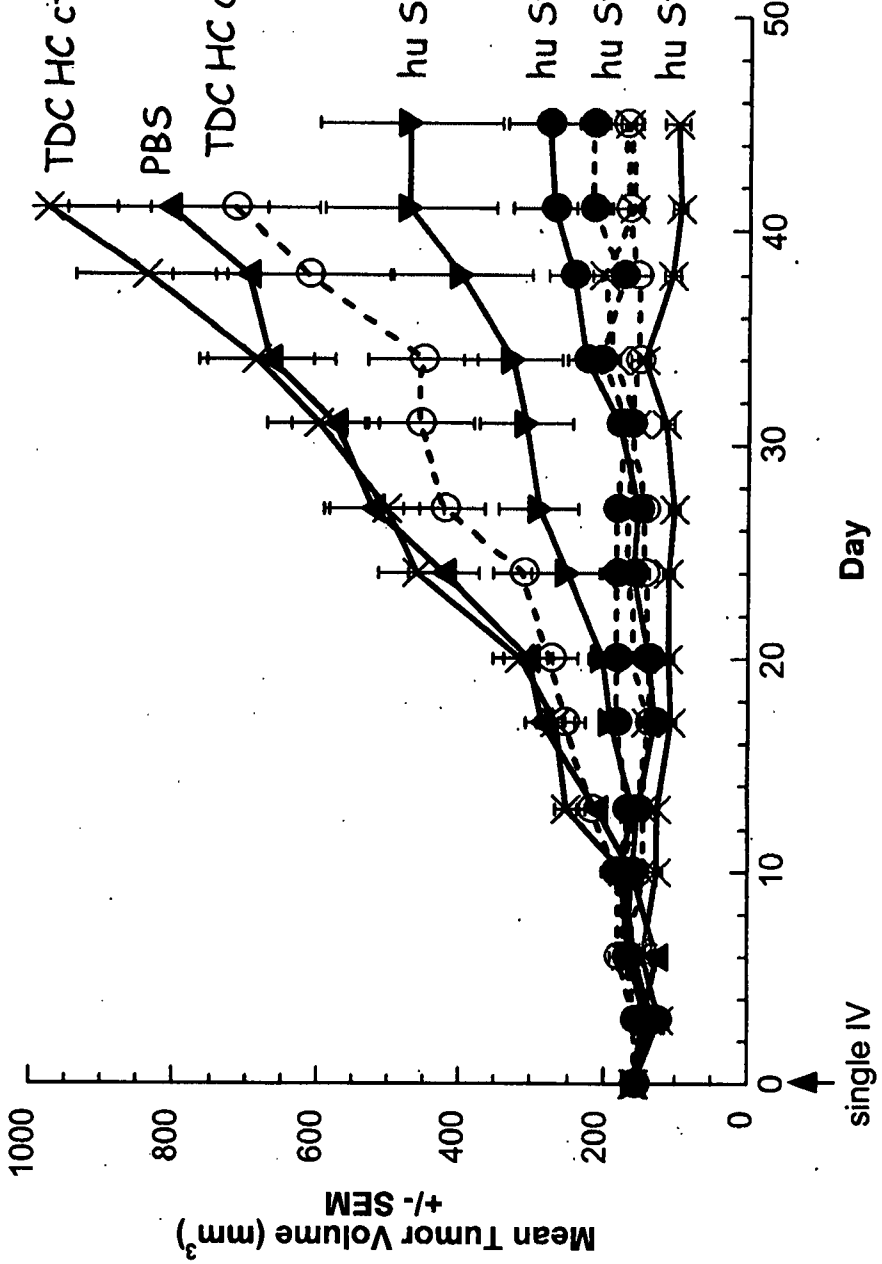
Fig. 19C

Figure 20



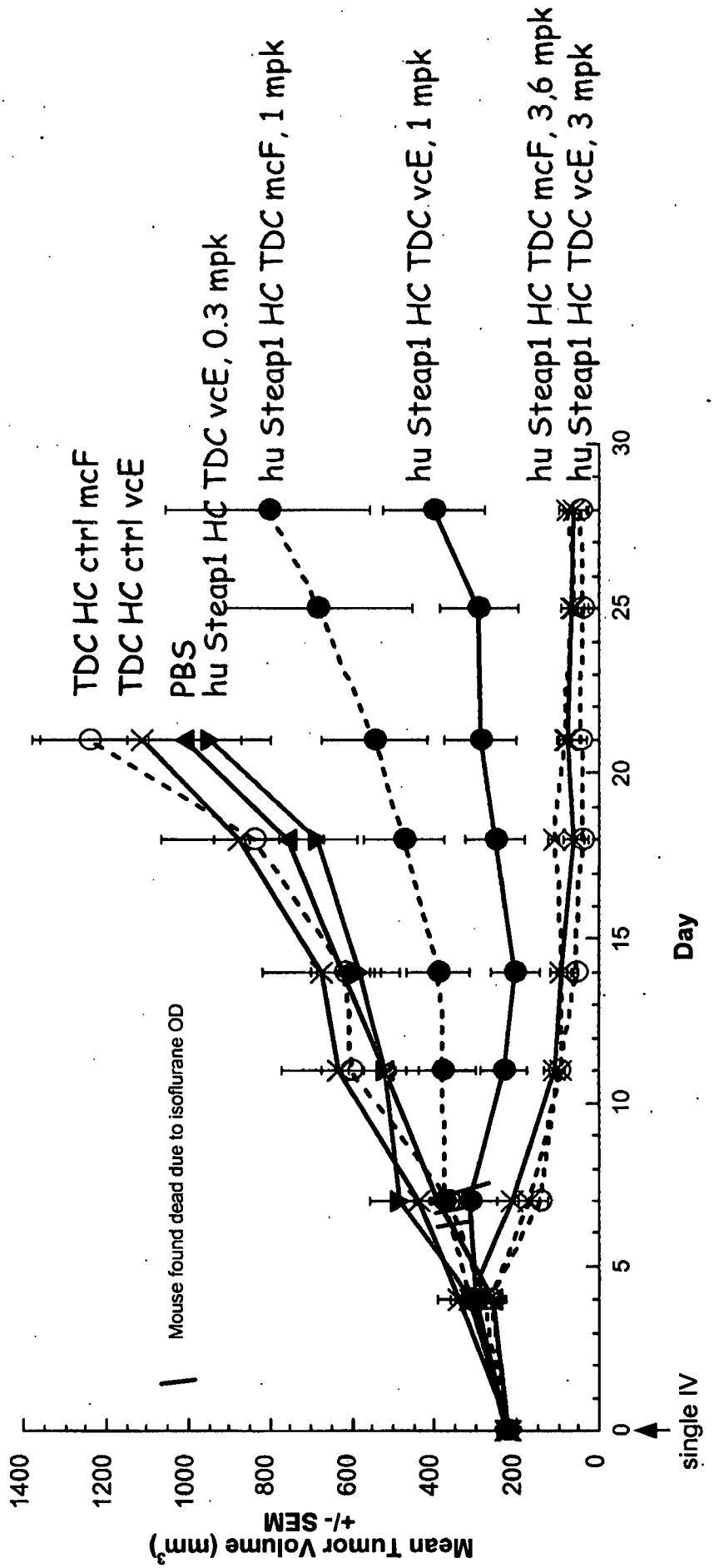
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Figure 21



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Figure 22



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Patente De Invención

09/349,904

5/5 09-34904

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2007/082726	26.10.2007	27.10.2006

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/30 A61K39/395 A61P35/00 C12N15/13 C12N5/10 A61K47/48 A61K51/10

Applicant
GENENTECH, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	see form PCT/ISA/210	Vadot, Pierre Telephone No. +31 70 340-3968

Form (PCT/ISA/237) (Cover Sheet) (April 2005)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☒ on paper

      ☒ in electronic form

   c. time of filing/furnishing:

      ☒ contained in the international application as filed.

      ☒ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

4. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 67-70,136,137,140-142,187,189-191

because:

☒ the said international application, or the said claims Nos. 67-70,136,137,140-142,187,189-191 relate to the following subject matter which does not require an international search *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☐ no international search report has been established for the whole application or for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-150,204-224
	No:	Claims	151-203
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-224
Industrial applicability (IA)	Yes:	Claims	1-66,71-135,138-140,143-186,188,192-224
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 67-70,136,137,140-142,187,189-191 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

2. No International Preliminary Examination will be carried out in respect of subject-matter which is not covered by the search report (Rule 66.1(e) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 2005/113601 A (AGENSYS INC [US]; JAKOBOVITS AYA [US]; ETESSAMI SOUDABEH [US]; CHALLIT) 1 December 2005 (2005-12-01)

D2: WO 2006/034488 A (GENENTECH INC [US]; EIGENBROT CHARLES W [US]; JUNUTULA JAGATH REDDY [U) 30 March 2006 (2006-03-30)

5.1 Novelty (Art. 33(2) PCT)

Independent claim 1 is novel since all the sequences are novel. Consequently, all the claims depending on claim 1 are also considered as novel.

Independent claim 12 is also novel for the same reason as for claim 1 (same sequences).

Independent claim 93 : in order to evaluate novelty of claim 93, a clarity problem must be first overcome since HVR-L3 has the same sequence as HVR-L1 : SEQ ID NO 11. But Fig. 2A shows that the sequence is different and consequently, HVR-L3 is defined by SEQ ID NO 13 : the search has been done with HVR-L3 being defined by sequences ID NO 13. Claim 93 is acknowledged as novel since even if SEQ ID NO 11

is already known (see SEQ ID NO 56 of WO2005113601), this sequence is not known in a cysteine engineered anti STEAP-1 antibody.

Independent claim 151 is not novel since SEQ ID NO 11 is not novel (see SEQ ID NO 56 of WO2005113601 which discloses an anti STEAP-1 antibody). All the claims 152-203 dependent on claim 151 are also considered as not novel.

Independent claims 204 and 206 dealing with SEQ ID N0: 90-101 are considered as novel.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 151-203 is not new in the sense of Article 33(2) PCT.

5.2 Inventive step (Art. 33(3) PCT)

Document D1 (WO2005113601) is considered as the closest prior art document.
The difference between D1 and the present application is that another antibody against STEAP-1 is claimed.
The effect of this difference is not known.
The problem can therefore be formulated as the provision of further alternative antibodies against STEAP-1.
The solution is found in claim 93 ie both of CDRs of the HC and LC are claimed in the same claim.
This solution cannot be acknowledged as inventive under art 33(3) PCT since a mere alternative of an antibody against a known antigen does not comprise the use of inventive skills.
The cysteine engineered anti-STEAP-1 antibody is a mere alternative form of antibody which is already well known in the antibody field (see document D2).

Unless the Applicant is able to show unexpected properties linked to the claimed antibody, the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-224 does not involve an inventive step in the sense of Article 33(3) PCT.

5.3 Claims 67-70,136,137,140-142,187,189-191 relate to a subject-matter considered

by this Authority to be covered by the provision of Rule 39.1(iv)/67.1(iv) PCT.
The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims 67-70,136,137,140-142,187,189-191 to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for in a first or further medical treatment.

Re Item VIII
Certain observations on the international application

Clarity, conciseness and support (Art. 6 EPC)

8.1 Claim 1 defines an antibody by its specificity and one, two or three HC of the HVRs. but it is clear through the examples that it is only an entire antibody (ie the 3 CDRs of the light chain AND the 3 CDRs of the heavy chain) which is responsible of the effect ie binding to STEAP-1. Therefore, claim 1 lacks of support and must be only defined by the association of both the three CDRs of the light and heavy chains. The same reasoning is kept to independent claims 151, 204, 206.

8.2 Claims 5 and 6 define the HC with at least 90% of identity to SEQ ID Nos: 9 or 10. It is well known in the antibody field that the change of only 1 amino acid in a sequence of an antibody can have detrimental effects such as the loss of the specificity. Therefore, the HC cannot be defined by a % of identity but only by the SEQ ID Nos 9 or 10.

8.3 Claim 15 refers back to claim 12 but should refer back to claim 14 dealing with a vector.

8.4 Dependent claims 15, 208 relate to the use of "host", therefore encompassing the use of cells originating from human embryos as well as human or animal body for industrial and/or commercial purposes.
Applicant's attention is drawn to the fact that, upon entry into the regional phase, patentability of claims relating to human embryos as well as human or animal body may underlie restrictions based on moral grounds. The EPO, for example, does not recognize as patentable the subject-matter of claims to the cloning of human beings, the modification of the germ line identity of human beings and the use of human embryos as well as the use of human or animal body for industrial or commercial purposes (Article 53(a) and Rule 23d EPC).

8.5 Claim 18 refers to a method of making an anti STEAP-1 antibody using the host cell of claim 15 which finally refers to claim 12 defining the HC-HVRs. For the same reasoning as in present objection 8.1, the method of making an antibody of claim 18 lacks of support : claim 18 only refers to one, or two or three HC HVRs instead of referring to three HC and HL HVRs. The same applies for claims 211.

8.6 Claim 19 refers to claim 1, thus further defining the antibody of claim 1. But as it stands, claim 19 says that STEAP-1 is expressed on the surface of a cell. This technical feature is useless for the definition of the antibody of claim 1 and on contrary, it brings unclarity : it seems that the antibody would be rather defined by its production process. Claims 19 and claims dependent thereon are not clear under Art. 6 PCT. The same objection applies for claim 212.

8.7 Claims 26 and 27 refer to claim 60; such clerical error must be corrected.

8.8 Claim 28 lacks of support since the method of detecting the present of STEAP-1 encompasses every kind of biological sample (ie urine) but it is clear that only sample from tissues is suitable since the antigen is a membrane antigen; it seems that no soluble antigen exists. A restriction to tissue sample is requested under Art. 6 PCT. The same objection is valid for claim 109,162,220.

8.9 Claim 67 generalises the use of the immunoconjugate to all cell proliferative disorders, but the examples are clearly limited to prostate cancer. The limitation must be accordingly done. The same objection applies to claims 136-137, 140-144, claims 189-193.

8.10 Claims 73 and 74 are defined with the help of unclear terms : " ... a thiol reactivity value..." and "... the cysteine engineered antibody is more reactive...". Indeed, the terms can be understood in many different ways since "reactive" is not linked to specific features, neither " a thiol reactivity value" (Art. 6 PCT). In the same way, claim 73 encompasses a process which seems to be needed to define the antibody of claim 1. Claim 75 further details the determination of the "thiol reactivity of the cysteine engineered antibody" whereas claim 73 already gives this value. This is not coherent : giving first the value and then way to determine this value (Art. 6 PCT).

8.11 Claim 87 : the term "antibody" is miswritten.

8.12 Claim 93 is not allowable since the antibody can be defined only by at least one HVR which is contrary to the reasoning given in objection 8.1. Further on, claim 93 is defined as a desiderata claim and is not allowable : indeed, only certain positions have been engineered to cysteins and it is well known that not every positions are available for such mutations as for instance all the positions of the CDRs (which allow the antibody to be defined). Therefore, a limitation to the mutations permitting the specificity of the antibody to be kept must be introduced (LC-V205C or HC-A118C or Fc-S400C) (Art. 6 PCT). The same reasoning applies for claims 96 and 98 (which depends on claim 1), and claim 152.

8.13 Claim 143 defines an article of manufacture. Surprisingly, it encompasses a compound that can be used to treat cancer characterized by the overexpression of a STEAP-1 polypeptide. The term "overexpression" seems to be essential since if STEAP-1 is not overexpressed, then the immunoconjugate is not active. Since this feature depends only on the antibody, the term "overexpression" must be present in each independent claims defining the antibody (Art. 6 PCT). the same applies to claim 192.

8.14 Claim 145 refers to claim 1 as being a cysteine engineered anti-STEAP-1 antibody, but claim 1 does not define a cysteine engineered anti STEAP-1 antibody. Claim 145 must be accordingly corrected.

8.15 Claim 164 lacks of support since the examples only rely on prostate cancer cell and are silent over B cell. The same applies to claims 186,187,189-191.

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

FORMULARIO ÚNICO DE CORRECCIONES Y MODIFICACIONES.
2000-10

① **SOLICITUD DE:**

☐ Corrección de Error Material. ☒ Modificación a una Solicitud.

② **SOLICITANTE**	Nombre: GENENTECH, INC., sociedad existente y organizada de conformidad con las leyes del Estado de Delaware, Estados Unidos de América. Dirección: 1 DNA Way, South San Francisco, CA 94080, Estados Unidos de América. Teléfono: 3 264270 Fax: 6 069701 E-mail: jllcco@lloredacamacho.com Domicilio: South San Francisco, Estado de California, Estados Unidos de América.	**IDENTIFICACION** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Número _____
③ **REPRESENTANTE O APODERADO**	Nombre: ALICIA LLOREDA RICAURTE Dirección: Calle 72 No. 5-83 5° Piso Teléfono: 3 264270 Fax: 6 069701 E-mail: jllcco@lloredacamacho.com	**IDENTIFICACION** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Número _____ TP _____

④ **Número de expediente:**
09-034.904

⑤ **Descripción de la corrección o modificación solicitada** Modificación capítulo Reivindicatorio y Descriptivo.

⑥ **Comprobante de pago No.** 09-34,259 **Fecha** Mayo 11 de 2009

⑦ **Anexos:**

☒ Comprobante de pago de la tasa.
☐ Poderes, si fuere el caso
☐ Certificado de existencia y representación legal de las partes, cuando sean personas jurídicas.

⑧ **NOMBRE** ALICIA LLOREDA **FIRMA** _____
C.C 39.690.713 53.215

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 09 - 34,259
FECHA : MAYO 11 DE 2009

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO DE BOGOTA	062754387	79464464	11/05/2009	19,590,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03 CAMBIO DE MODALIDAD Y MODIFICA	12 MARCAS Y LEMAS COMERCIALES	111,000.00
		TOTAL : $	111,000.00

SON: CIENTO ONCE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

RESUMEN, OBJETO Y FINALIDAD DE LA INVENCION

ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

Se proporcionan anticuerpos anti-STEAP-1 y sus inmunoconjugados. Se proporcionan métodos de uso de los anticuerpos anti-STEAP-1 y de sus inmunoconjugados.

ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

CAMPO DE LA INVENCIÓN

[0001] La presente invención se relaciona con los anticuerpos anti-STEAP-1 y sus inmunoconjugados. La invención se refiere además, a los métodos de utilización de los anticuerpos anti-STEAP-1 y de sus inmunoconjugados.

ANTECEDENTES

[0002] En los seres humanos, el cáncer de próstata es uno de los tumores malignos de diagnóstico más común en los hombres; ocupa el segundo lugar entre las principales causas de muerte por cáncer en los hombres. Según las estimaciones de la American Cancer Society, durante el año 2000 se diagnosticaron 180.400 casos nuevos de cáncer de próstata con el resultado de 31.900 muertes a causa de la enfermedad. En las fases avanzadas, el cáncer de próstata provoca metástasis óseas. Aunque se han logrado avances en el diagnóstico precoz y el tratamiento de los tumores localmente limitados, el cáncer de próstata es incurable una vez que ha hecho metástasis. Los pacientes con cáncer de próstata metastásico sometidos a terapia hormonal terminarán por desarrollar un estado refractario al andrógeno (andrógeno independiente) que conducirá a la progresión de la enfermedad y a la muerte. En la actualidad, el antígeno específico de la próstata (PSA) es el marcador tumoral de uso más generalizado para la exploración, el diagnóstico y el control del cáncer de próstata. No obstante, el uso generalizado del PSA como una herramienta para la exploración es controvertido puesto que el PSA no logra discriminar exactamente entre tumor prostático benigno y maligno.

[0003] Según la fase del cáncer, el tratamiento contra el cáncer de próstata y de vejiga implica una de las siguientes terapias o una combinación de las mismas: cirugía para extirpar el tejido canceroso, radioterapia, quimioterapia, privación de andrógeno (p.ej., terapia hormonal) en el caso del cáncer de próstata. Aunque la cirugía y la radioterapia mejoran significativamente la supervivencia en los pacientes que se hallan en las primeras fases de la enfermedad, las opciones terapéuticas son muy limitadas cuando se trata de casos avanzados, en particular en la recurrencia de tumores después de la supresión hormonal. La mayoría de los pacientes sometidos a terapia hormonal evolucionan hacia el desarrollo de un tumor andrógeno independiente. En la actualidad no existe ningún tratamiento eficaz para el 20-40% de los pacientes con cáncer de próstata que desarrollan un tumor recurrente después de la cirugía o la radioterapia, o para quienes presentaban un cáncer con metástasis en el momento del diagnóstico. La quimioterapia tiene efectos secundarios tóxicos, especialmente en los pacientes de mayor edad. El desarrollo de nuevas formas de terapia, especialmente para los tumores refractarios a la privación de andrógeno, es una necesidad urgente para el tratamiento del carcinoma prostático.

[0004] Ha sido descrita (véase Patente de EE.UU. Nº 6.329.503) la identificación un nuevo antígeno de superficie celular, STEAP-1. STEAP-1 forma parte de los antígenos de superficie celular de dominio transmembrana que configuran una serpentina. Se expresa predominantemente en el cáncer de próstata y por ese motivo los miembros de esa familia reciben la denominación de "STEAP" (Six Transmembrane Epithelial Antigens of the Prostate – Seis antígenos epiteliales transmembranos de la próstata). Las proteínas STEAP humanas presentan un elevado grado de conservación estructural dentro de la familia, pero no muestran ninguna homología estructural significativa con ninguna proteína conocida. La STEAP-1 parece ser un tipo de proteína IIIa de membrana que se expresa predominantemente en las células prostáticas en tejidos humanos normales. Estructuralmente, la STEAP-1 es una proteína aminoácida 339 caracterizada por una topología molecular de seis dominios de transmembrana y terminales intracelulares N- y C-, lo cual indica que se enrolla en forma de "serpentina" en tres bucles extracelulares y dos intracelulares. La expresión de la proteína STEAP-1 se mantiene a niveles elevados a través de los distintos estados del cáncer de próstata. La STEAP-1 tiene una sobreexpresión elevada en otros cánceres humanos, como el de pulmón y el de colon. Se cultivaron anticuerpos murinos para los fragmentos de STEAP-1 humanos y los anticuerpos mostraron unirse a la STEAP-1 sobre la superficie celular (véase Solicitud de Patente de EE.UU. Nº 20040253232A1).

[0005] La terapia basada en anticuerpos se ha revelado muy eficaz en el tratamiento de distintos tipos de cáncer. Por ejemplo, HERCEPTIN® y RITUXAN® (ambos de Genentech Inc., South San Francisco, California) son anticuerpos que se han utilizado con éxito para el tratamiento del cáncer de mama y del linfoma no Hodgkin, respectivamente. HERCEPTIN® es un anticuerpo monoclonal humanizado derivado de ADN recombinante que se une selectivamente al dominio extracelular del protooncogén del receptor 2 del factor de crecimiento epidérmico humano (HER2). En el 25-30% de los cánceres primarios de mama se observa una sobreexpresión de la proteína del HER2. RITUXAN® es un anticuerpo monoclonal quimérico humano/murino creado mediante ingeniería genética y dirigido contra el antígeno CD20 que se encuentra en la superficie de linfocitos B malignos y normales. Estos dos anticuerpos se producen en células CHO.

[0006] El uso de anticuerpos conjugados con fármacos para la administración local de agentes citotóxicos o citostáticos, es decir, los fármacos que se utilizan para matar o inhibir células tumorales en el tratamiento del cáncer (Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz y Springer (1997) *Adv. Drg Del. Rev.* 26: 151-172; patente de EE. UU. n.º 4975278), permite la administración de la fracción de fármacos a tumores y una acumulación intracelular en éstos, donde la administración sistemática de estos agentes farmacológicos no conjugados puede resultar en niveles inaceptables de toxicidad tanto para las células normales como para las células tumorales que se intentan eliminar (Baldwin y col., (1986) *Lancet* págs. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers

Of Cytotoxic Agents In Cancer Therapy: A Review," en *Monoclonal Antibodies '84: Biological And Clinical Applications*, A. Pinchera y col. (eds), págs. 475-506). Se busca entonces la máxima eficacia con la mínima toxicidad. Tanto los anticuerpos monoclonales como los policlonales se han descrito como útiles en estas estrategias (Rowland y col., (1986) *Cancer Immunol. Immunother.*, 21:183-87). Los fármacos empleados en estos métodos incluyen a la daunomicina, la doxorrubicina, el metotrexato y la vindesina (Rowland y col., *Cancer Immunol. Immunother.* 21:183-87 (1986)). Entra las toxinas empleadas en anticuerpos conjugados con toxinas se encuentran las toxinas bacterianas como la toxina de la difteria, toxinas vegetales como la ricina, toxinas de pequeñas moléculas como la geldanamicina (Kerr y col., (1997) *Bioconjugate Chem.* 8(6):781-784; Mandler y col., (2000) *Journal of the Nat. Cancer Inst.* 92(19):1573-1581; Mandler y col., (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler y col., (2002) *Bioconjugate Chem.* 13:786-791), maitansinoides (patente europea n.º EP 1391213; Liu y col., (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623) y caliqueamicina (Lode y col. (1998) *Cancer Res.* 58:2928; Hinman y col., (1993) *Cancer Res.* 53:3336-3342). Las toxinas pueden producir sus efectos citotóxicos y citostáticos mediante mecanismos que incluyen la unión de tubulina, la unión de ADN o la inhibición de la topoisomerasa (Meyer, D.L. y Senter, P.D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" en *Annual Reports in Medicinal Chemistry*, Vol 38 (2003) Capítulo 23, 229-237). Algunos fármacos citotóxicos tienden a ser inactivos o menos activos cuando se conjugan con grandes anticuerpos o ligandos receptores de proteínas.

[0007] ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) es un anticuerpo conjugado con un radioisótopo compuesto de un anticuerpo monoclonal kappa IgG1 murino dirigido contra el antígeno CD20 que se encuentra en la superficie de los linfocitos B cancerígenos y normales, y contra el radioisótopo ^{111}In o ^{90}Y unido por un quelante enlazador de tiourea (Wiseman y col., (2000) *Eur. Jour. Nucl. Med.* 27(7):766-77; Wiseman y col., (2002) *Blood* 99(12):4336-42; Witzig y col., (2002) *J. Clin. Oncol.* 20(10):2453-63; Witzig y col., (2002) *J. Clin. Oncol.* 20(15):3262-69). A pesar de que el ZEVALIN presenta actividad contra las células B del linfoma no Hodgkin (LNH), su administración provoca citopenias graves y prolongadas en la mayoría de los pacientes. MYLOTARG™ (Gentuzumab ozogamicina, Wyeth Pharmaceuticals), un conjugado de anticuerpos y fármacos compuesto de un anticuerpo huCD33 enlazado a caliqueamicina, se aprobó en 2000 para el tratamiento de leucemia mieloide aguda por inyección (*Drugs of the Future* (2000) 25(7): 686; Patentes de EE.UU. N.º 4.970.198; 5.079.233; 5.585.089; 5.606.040; 5.693.762; 5.739.116; 5.767.285; 5.773.001). Cantuzumab mertansina (Inmugen, Inc.), un anticuerpo conjugado con un fármaco compuesto por el anticuerpo huC242 enlazado a la fracción de fármacos de los maitansinoides por medio de un enlazado de disulfuro SPP, DM1, está desarrollado para el tratamiento de cánceres que expresan el antígeno CanAg, tales como los de colon, páncreas, gástricos y otros. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), un anticuerpo conjugado con un fármaco, compuesto por el anticuerpo monoclonal del antígeno de membrana antiprostático (PSMA) enlazado al

grupo de los maitansinoides, DM1, está en fase de desarrollo para el tratamiento potencial de los tumores de próstata. La misma fracción de fármacos de los maitansinoides, DM1, se unió mediante un enlazador no disulfuro, SMCC, a un anticuerpo monoclonal murino, TA.1 (Chari y col., (1992) *Cancer Research* 52:127-131). Se informó que dicho conjugado es 200 veces menos potente que el correspondiente conjugado del enlazador de disulfuro. En la presente memoria descriptiva, el enlazado SMCC se consideró "no escindible".

[0008] Se aislaron varios compuestos peptídicos cortos del molusco marino, Dolabella auricularia, y se descubrió que tenían actividad biológica (Pettit y col., (1993) *Tetrahedron* 49:9151; Nakamura y col., (1995) *Tetrahedron Letters* 36:5059-5062; Sone y col., (1995) *Journal Org Chem* 60:4474). También se prepararon análogos de estos compuestos y se descubrió que algunos tenían actividad biológica (para su reseña, véase Pettit y col., (1998) *Anti-Cancer Drug Design* 13:243-277). Por ejemplo, la auristatina E (patente de EE.UU. N.º 5635483) es un análogo sintético del producto natural marino dolastatina 10, un agente que inhibe la polimerización de la tubulina al unirse al mismo punto en la tubulina que en la vincristina con acción antineoplásica (G. R. Pettit, (1997) *Prog. Chem. Org. Nat. Prod.* 70:1-79). La dolastina 10, auristatina PE y auristatina E son péptidos lineales que tienen cuatro aminoácidos, tres de lo cuales son únicos de la clase de compuestos de la dolastina, y una amida C-terminal.

[0009] Los péptidos auristatina, la auristatina E (AE) y la monometilauristatina (MMAE), análogos sintéticos de la dolastatina, se conjugaron a: (i) anticuerpos monoclonales quiméricos cBR96 (específicos para Lewis Y en carcinomas); (ii) cAC10 que es específico para CD30 en tumores hematológicos (Klussman, y col., (2004), *Bioconjugate Chemistry* 15(4):765-773; Doronina y col., (2003) *Nature Biotechnology* 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco y col., (2003) *Blood* 102(4):1458-1465; patente de EE.UU. N. º 2004/0018194; (iii) anticuerpos anti-CD20 tal como Rituxan® (rituximab) (documento WO 04/032828) para el tratamiento de células tumorales que expresan CD20 y trastornos inmunitarios; (iv) anticuerpos 2H9 anti-EphB2 y anti-IL-8 para el tratamiento del cáncer colorrectal (Mao, y col., (2004) *Cancer Research* 64(3):781-788); (v) anticuerpo E-selectina (Bhaskar y col., (2003) *Cancer Res.* 63:6387-6394) y (vi) otros anticuerpos anti-CD30 (documento WO 03/043583). La monometilauristatina (MMAE) también se conjugó con 2H9, un anticuerpo contra EphB2R que es un receptor de tirosina quinasa TM tipo 1 con una homología cercana entre el ratón y el humano, y se sobreexpresa en las células cancerígenas colorrectales (Mao y col., (2004) *Cancer Res.* 64:781-788).

[0010] Se informó que la monometilauristatina MMAF, una variante de la auristatina E (MMAE) con una fenilalanina en C-terminal (patente de EE.UU. N.º 5767237; patente de EE.UU. N.º 6124431), es menos potente que la MMAE, sin embargo, es más potente cuando se conjuga con anticuerpos monoclonales (Senter y col., *Proceedings for the American Association for Cancer Research*, Volumen 45,

Abstract Number 623, presentado el 28 de marzo de 2004). El fenileno diamina auristatina F (AFP), una variante fenilalanina de MMAE que se unió al anti-CD70 mAb, 1F6, a través del C-terminal de 1F6 mediante un separador de fenileno diamina (Law y col., *Proceedings of the American Association for Cancer Research*, Volumen 45, Abstract Number 625, presentado el 28 de marzo de 2004).

[0011] En la materia existe la necesidad de otros fármacos para tratar diversos cánceres, como por ejemplo cánceres y metástasis de cánceres en la próstata, pulmón y colon. Los fármacos especialmente útiles para este fin incluyen los conjugados de anticuerpos anti-STEAP-1 con fármacos que actúan sobre las células de próstata, pulmón o colon y que tienen una toxicidad considerablemente baja per con con una eficacia terapéutica útil. Éstas y otras limitaciones y problemas del pasado se tratan en la presente invención.

[0012] El recitado de las referencias en la presente solicitud no representa una admisión de que la referencia es un conocimiento en la materia anterior a esta solicitud. Todas las referencias citadas en la presenta memoria descriptiva, incluyendo las patentes, las solicitudes de patentes y publicaciones, quedan incorporadas únicamente a modo de referencia.

RESUMEN

[0013] La invención proporciona anticuerpos anti-STEAP-1 y sus métodos de utilización.

[0014] En un aspecto, se proporciona un anticuerpo que se une a STEAP-1, en el que el anticuerpo comprende un dominio variable de cadena ligera que comprenda la secuencia de aminoácidos mostrada en la Figura 2A (identificador de secuencia n.º: 6 - ID. SEC. N°6) o un dominio variable de cadena pesada que comprenda la secuencia de aminoácidos mostrada en la Figura 2B (identificador de secuencia n.º: 9 - ID. SEC. N°9). En un aspecto, se proporciona un anticuerpo que se une a STEAP-1, en el que el anticuerpo comprende un dominio variable de la cadena ligera que comprenda la secuencia de aminoácidos mostrada en la Figura 2A (identificador de secuencia n.º: 6) y un dominio variable de cadena pesada que comprenda la secuencia de aminoácidos que se muestra en la Figura 2B (identificador de secuencia n.º: 9)

[0015] En un aspecto, se proporciona un anticuerpo que se une a STEAP-1, en el que el anticuerpo comprende un dominio variable de la cadena pesada con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98 ó 99% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º 9 ó 10. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena pesada de identificador de secuencia n.º 9 ó 10. En una realización, el anticuerpo comprende una región estructural de dominio variable de cadena pesada 1 de ID. SEC. Nº 25 o una región estructural de dominio variable de cadena pesada 2 de ID. SEC. Nº 75 ó 76 ó 77 o una región estructural de dominio variable de cadena pesada 3 de ID. SEC. Nº 78 ó 79.

[0016] En un aspecto, el anticuerpo comprende un dominio variable de la cadena ligera con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98 ó 99% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º 6. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena ligera de identificador de secuencia n.º 6.

[0017] En un aspecto, se ofrece un anticuerpo que se une a STEAP-1, en donde el anticuerpo comprende un dominio variable de cadena pesada que tiene al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, al menos 95%, al menos 96%, al menos 97%, al menos 98% o al menos 99% de la identidad de secuencia de una secuencia de aminoácido de ID. SEC. Nº 9 ó 10. En una realización el anticuerpo comprende un dominio variable de cadena pesada de ID. SEC. Nº 9 ó 10. En una realización, el anticuerpo comprende una región estructural de dominio variable de cadena pesada 1 de ID. SEC. Nº 25 o una región estructural de dominio variable de cadena pesada 2 de ID. SEC. Nº 75 ó 76 ó 77 o una región estructural de dominio variable de cadena pesada 3 de ID. SEC. Nº 78 ó 79. En una forma de realización, el anticuerpo también comprende un dominio variable de la cadena ligera con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98 ó 99% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º 6. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena ligera de identificador de secuencia n.º 6.

[0018] En ciertas formas de realización, se proporciona un polinucleótido que codifica cualquiera de los anticuerpos anteriores. En una forma de realización, se proporciona un vector que comprende el polinucleótido. En una forma de realización, se proporciona un anfitrión celular que comprende el vector. En una forma de realización, el anfitrión celular es una célula eucariótica. En una forma de realización, el anfitrión celular es una célula de ovario de hámster chino (CHO). En una forma de realización, se proporciona un método de fabricación de un anticuerpo anti-STEAP-1, en la que el método comprende el cultivo del anfitrión celular en condiciones aptas para la expresión del polinucleótido que codifica el anticuerpo y el aislamiento del anticuerpo.

[0019] En un aspecto, se proporciona un anticuerpo que se une a STEAP-1 expresado en la superficie de una célula. En una forma de realización, el anticuerpo se une a un epítopo en la región del STEAP-1 humano o de ratón. En una forma de realización, la célula es una célula mamífera. En una forma de realización, la célula es una célula humana. En una forma de realización, la célula es una célula cancerosa. En una forma de realización, la célula es una célula de próstata, pulmón o colon. En una forma de realización, la célula cancerosa es una célula de cáncer de próstata. En otra realización, la célula procede de una metástasis de un cáncer primario de próstata, pulmón o colon.

[0020] En ciertas formas de realización, cualquiera de los anticuerpos anteriores es un anticuerpo monoclonal. En una forma de realización, el anticuerpo es un fragmento de anticuerpo seleccionado entre

el fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$. En una forma de realización, el anticuerpo es humanizado. En una forma de realización, el anticuerpo es humano.

[0021] En un aspecto, se proporciona un método de detección de la presencia de STEAP-1 en una muestra biológica y dicho método comprende establecer el contacto de la muestra biológica con cualquiera de los anticuerpos anteriores en condiciones que faciliten la unión del anticuerpo a STEAP-1 y detectar si un complejo está formado por el anticuerpo y STEAP-1. En una forma de realización, la muestra biológica comprende las células de próstata. En una forma de realización, la muestra biológica se obtiene de un mamífero que experimenta o se cree que experimenta un trastorno de células de próstata o un trastorno proliferativo de células o tejidos, o ambos, incluyendo, entre otros, cáncer de próstata, pulmón, colon, vejiga y ovario y sarcoma de Ewing, así como, metástasis cánceres primaries de próstata, pulmón, colon, vejiga y ovario y sarcoma de Ewing. Véase, por ejemplo, (véase la patente de EE.UU. nº 6.329.503; y Rodeberg, D.A. y col., Clin. Cancer Res. 11(12):4545-4552 (2005)).

[0022] En un aspecto, se proporciona un método de diagnóstico de un trastorno de proliferación celular asociado a un aumento de la expresión de STEAP-1. Este método comprende establecer un contacto entre una célula de prueba y cualquiera de los anticuerpos anteriores, determinar el nivel de expresión de STEAP-1 mediante la detección de la unión del anticuerpo a STEAP-1 y comparar el nivel de expresión de STEAP-1 mediante la célula de prueba con el nivel de expresión de STEAP-1 mediante una célula de control, donde un nivel más elevado de expresión de STEAP-1 mediante la célula de prueba frente a la célula de control indica la presencia de un trastorno de proliferación celular asociado a un aumento de la expresión de STEAP-1. En una forma de realización, la célula de prueba es una célula de un paciente que se cree que padece un trastorno de proliferación célular tal como el trastorno proliferativo de próstata. En una forma de realización, el trastorno proliferativo se selecciona a partir de los trastornos de células de próstata que incluyen, entre otros, cáncer de próstata. En una forma de realización, el método comprende la determinación del nivel de expresión de STEAP-1 en la superficie de la célula de prueba (como, por ejemplo, una célula de cancer de próstata) y la comparación del nivel de expresión de STEAP-1 en la superficie de la célula de prueba con el nivel de expresión de STEAP-1 en la superficie de la célula de control (como, por ejemplo, una célula de próstata normal distinta de una célula de próstata anormalmente proliferativa).

[0023] En un aspecto, se proporciona un método de diagnóstico de un trastorno de proliferación celular asociado a un aumento de células, tales como las células próstata, que expresan STEAP-1. Este método comprende establecer un contacto entre células de prueba en una muestra biológica y cualquiera de los anticuerpos anteriores, determinar el nivel de unión del anticuerpo a células de prueba en la muestra mediante la detección de la unión del anticuerpo a STEAP-1 y comparar el nivel de unión del anticuerpo a las células en una muestra de células de control, donde el nivel de unión del anticuerpo se normaliza a la

cantidad de células que expresan STEAP-1 en las muestras de células de prueba y de control, y en donde el nivel más elevado de unión del anticuerpo en la muestra de células de prueba comparado con la muestra de células de control indica la presencia de un trastorno de proliferación celular asociado a células que expresan STEAP-1.

[0024] En un aspecto, se proporciona un método de detección de STEAP-1 soluble en la sangre o el suero. Este método comprende establecer el contacto entre una muestra de prueba de sangre o suero de un mamífero que se cree que experimenta un trastorno proliferativo de células próstata con un anticuerpo anti-STEAP-1 de la invención, y detectar un aumento en el STEAP-1 soluble en la muestra de prueba con respecto a la muestra de control de sangre o suero de un mamífero normal. En una forma de realización, el método de detección es útil como método de diagnóstico de trastornos proliferativos de células próstata asociados a un aumento de STEAP-1 soluble en la sangre o el suero de un mamífero.

[0025] En un aspecto, los anticuerpos de la invención incluyen anticuerpos diseñados con cisteína en donde se reemplazan uno o más aminoácidos de un anticuerpo parental con un aminoácido de cisterna libre, tal como se describe en el documento WO2006/034488 (íntegramente incorporado en la presente memoria descriptiva a modo de referencia). Un anticuerpo diseñado con cisteína comprende uno o más aminoácidos de cisteína libre con un valor de reactividad del tiol en un rango de entre 0,6 y 1,0. Un aminoácido de cisteína libre es un residuo de cisteína que se ha diseñado en anticuerpo parental y no forma parte de un enlace disulfuro. Los anticuerpos diseñados con cisteína son útiles para la unión de compuestos citotóxicos o de imágenes, o ambos, en una posición de la cisteína diseñada mediante, por ejemplo, una maleimida o un haloacetil. La reactividad nucleofílica de la funcionalidad tiol de un residuo cis con un grupo maleimido es aproximadamente 1000 veces mayor en comparación con cualquier otra funcionalidad aminoácida en una proteína, como por ejemplo, el grupo amino de residuos de lisina o el grupo amino N terminal. La funcionalidad tiol específica en los reactivos iodoacétilos y maleimidos puede reaccionar con grupos aminos, sin embargo, se requieren tiempos de reacción mayores a pH (>9,0) y más prolongados (Garman, 1997, *Non-Radioactive Labelling*: *A Practical Approach*, Academic Press, London).

[0026] Los anticuerpos diseñados con cisteína pueden ser útiles en el tratamiento del cáncer e incluir anticuerpos específicos para la superficie celular y los receptores transmembranales, y antígenos asociados al tumor (AAT). Estos anticuerpos pueden utilizarse como anticuerpos desnudos (no conjugados con una fracción de fármacos o marcas) o como anticuerpos conjugados con fármacos (ADC). Los anticuerpos de la invención diseñados con cisteína pueden ubicarse específicamente o acoplarse eficazmente con un reactivo del tiol. El reactivo del tiol puede ser un reactivo enlazador multifuncional, un reactivo de captura marcado, un reactivo fluoróforo o un enlazador a fármacos intermedio. El anticuerpo diseñado con cisteína puede marcarse con una marca detectable, inmobilizarse en un soporte de fase sólida o conjugarse con una fracción de fármacos, o todas las anteriores. La reactividad del tiol puede

generalizarse en cualquier anticuerpo, donde la sustitución de aminoácidos por aminoácidos de cisteínas reactivas puede realizarse dentro de los rangos en la cadena ligera seleccionados a partir de los rangos de aminoácidos: de L-10 a L-20, de L-38 a L-48, de L-105 a L-115, de L-139 a L-149, de L-163 a L-173; y dentro de los rangos en la cadena pesada seleccionados a partir de los rangos de aminoácidos: de H-35 a H-45, de H-83 a H-93, de H-114 a H-127 y de H-170 a H-184; y en la región Fc, dentro de los rangos selecionados de H-268 a H-291, de H-319 a H-344, de H-370 a H-380 y de H-395 a H-405, en el que la numeración de las posiciones de los aminoácidos comienza a partir de la posición 1 del sistema de numercación de Kabat (Kabat y col., (1991) *Sequences of Proteins of Immunological Interest*, 5.ª Ed. Public Health Service, National Institutes of Health, Bethesda, MD) y continúa secuencialmente a partir de entonces, tal como se describe en el documento WO 2006/034488. La reactividad del tiol también puede generalizarse en determinados dominios de un anticuerpo, como por ejemplo, el dominio constante de la cadena ligera (CL) y los dominios constantes de la cadena pesada, CH1, CH2 y CH3. Las sustituciones de cisteínas que producen como resultado los valores de reactividad del tiol de 0,6 y mayores pueden realizarse en los dominios constantes de la cadena pesada α, δ, ϵ, γ y μ de anticuerpos intactos: IgA, IgD, IgE, IgG e IgM, respectivamente, incluyendo las subclases IgG: IgG1, IgG2, IgG3, IgG4, IgA e IgA2. Estos anticuerpos y sus usos se describen en el documento WO 2006/034488.

[0027] Los anticuerpos de la invención diseñados con cisteína conservan preferentemente la capacidad de unión al antígeno de los homólogos anticuerpos parentales de tipo natural. Por lo tanto, los anticuerpos diseñados con cisteína son capaces de unirse específica y preferentemente con antígenos. Dichos antígenos incluyen, por ejemplo, antígenos asociados al tumor (AAT), proteínas de los receptores de la superficie celular y otras moléculas de la superficie celular, proteínas transmembranas, proteínas de señalización, factores reguladores de la supervivencia celular, factores reguladores de la proliferación celular, moléculas asociadas al (por ejemplo, que se sabe o se cree que contribuyen de manera funcional al) desarrollo o diferenciación del tejido, linfoquinas, citoquinas, moléculas implicadas en la regulación del ciclo celular o en la vasculogénesis y moléculas asociadas a (por ejemplo, que se sabe o se cree que contribuyen de manera funcional a) la angiogénesis.

[0028] Un anticuerpo de la invención puede conjugarse a otros agentes reactivos del tiol en los que el grupo reactivo es, por ejemplo, un maleimido, una iodoacetamida, un piridil disulfuro u otra molécula de conjugación de los reactivos del tiol (Haugland, 2003, *Molecular Probes Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc.; Brinkley, 1992, *Bioconjugate Chem.* 3:2; Garman, 1997, *Non-Radioactive Labelling: A Practical Approach,* Academic Press, London; Means (1990) *Bioconjugate Chem.* 1:2; Hermanson, *G.* en *Bioconjugate Techniques* (1996) Academic Press, San Diego, págs. 40-55, 643-671). La molécula puede ser un agente citotóxico (por ejemplo, una toxina tal como la doxorrubicina o toxina pertussis), un fluoróforo tal como un tinte fluorescente como la fluoresceína o rodamina, un agente

quelante de metales para la radioterapia o en diagnóstico por imágenes, una marca peptidil o no peptidil o una marca de detección, o un agente modificador de la eliminación como por ejemplo muchos isomeros del polietilenglicol, un péptido que se une a un tercer componente u otro carbohidrato o agente lipofílico.

[0029] En un aspecto, los anticuerpos de la invención pueden conjugarse con cualquier grupo de marcas que pueda unirse covalentemente al anticuerpo a través de un grupo reactivo, un grupo activo o un grupo tiol de cisteína reactivo (Singh y col., (2002) *Anal. Biochem.* 304:147-15; Harlow E. y Lane, D. (1999) *Using Antibodies*: *A Laboratory Manual*, Cold Springs Harbor Laboratory Press, Cold Spring Harbor, NY; Lundblad R.L. (1991) *Chemical Reagents for Protein Modification*, 2.ª ed. CRC Press, Boca Raton, FL). La marca unida puede: (i) proporcionar una señal detectable; (ii) interactuar con una segunda marca para modificar la señal detectable que proporcionó la primera o la segunda marca, por ejemplo, para brindar FRET (transferencia resonante de energía de fluorescencia); (iii) estabilizar las interacciones o aumentar la afinidad de unión con un antígeno o ligando; (iv) afectar la movilidad, como por ejemplo, la movilidad electroforética o la permeabilidad celular mediante la carga, hidrofobicidad, forma u otros parámetros físicos; o (v) proporcionar el grupo de captura para modular la afinidad del ligando, la unión antígeno-anticuerpo o la formación de complejos iónicos.

[0030] Los anticuerpos diseñados con cisteína y marcados pueden ser útiles en los ensayos de diagnóstico, por ejemplo, para detectar la expresión de un antígeno de interés en células, tejidos o sueros específicos. Para las aplicaciones de diagnóstico, el anticuerpo generalmente se marcará con un grupo detectable. Se dispone de numerosas marcas que pueden agruparse, por lo general, en las siguientes categorías:

[0031] Radioisotopos (radionucleidos) tales como ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At o ^{213}Bi. Los anticuerpos marcados con radioisotopos son útiles en los experimentos de diagnóstico por imágenes que actúan sobre el receptor. El anticuerpo puede marcarse con reactivos ligandos que unen agentes quelantes de metales u otros complejos para el radioisótopo, donde el agente es reactivo con el tiol diseñado con cisteína del anticuerpo, mediante las técnicas que se describen en *Current Protocols in Immunology*, Volúmenes 1 y 2, Coligen y col., Ed. Wiley-Interscience, New York, NY, Pubs. (1991). Los ligandos quelantes que pueden combinar un metal ion incluyen DOTA, DOTP, DOTMA, DTPA y TETA (Macrocyclics, Dallas, TX). Los radionucleidos pueden indicarse a través de la formación de complejos con anticuerpos de la invención conjugados con fármacos (Wu y col., (2005) *Nature Biotechnology* 23(9):1137-1146).

[0032] Los reactivos enlazadores tales como el maleimido-DOTA (4-maleimidobutiramidobenzilo-DOTA) pueden prepararse mediante la reacción del aminobenzilo-DOTA con el ácido 4-maleimidobutirico (Fluka) activado con formatocloroisopropil (Aldrich), siguiendo el procedimiento de Axworthy y col., (2000) *Proc. Natl. Acad. Sci. USA* 97(4):1802-1807). Los agentes

reactivos maleimido-DOTA reaccionan con los aminoácidos de cisteína libre de los anticuerpos diseñados con cisteína y proporcionan un ligando de formación de complejos de metal en el anticuerpo (Lewis y col., (1998) *Bioconj. Chem.* 9:72-86) Los reactivos de marcación con enlazadores quelantes tales como DOTA-NHS (1, 4, 7, 10-tetraazaciclododecano- 1, 4, 7, 10-monoácido tetraacético (éster de N-hidroxisuccinimida) se encuentran comercialmente disponibles (Macrocyclics, Dallas, TX). La imagen que actúa sobre el receptor con los anticuerpos marcados con radionucleidos puede proporcionar un marcador de una vía de activación mediante la detección y cuantificación de la acumulación progresiva de anticuerpos en el tejido tumoral (Albert y col., (1998) *Bioorg. Med. Chem. Lett.* 8:1207-1210). Los radiometales conjugados pueden permanecer intracelulares después de la degradación lisososmal.

[0033] Los complejos quelantes de metales que sirven como marcas de anticuerpos para los experimentos de diagnóstico por imágenes se describen en: patente de EE.UU. N.º 5.342.606; patente de EE.UU. N.º 5.428.155; patente de EE.UU. N.º 5.316.757; patente de EE.UU. N.º 5.480.990; patente de EE.UU. N.º 5.462.725; patente de EE.UU. N.º 5.428.139; patente de EE.UU. N.º 5.385.893; patente de EE.UU. N.º 5.739.294; patente de EE.UU. N.º 5.750.660; patente de EE.UU. N.º 5.834.456; Hnatowich y col., (1983) *J. Immunol. Methods* 65:147-157; Meares y col., (1984) *Anal. Biochem.* 142:68-78; Mirzadeh y col., (1990) *Bioconjugate Chem.* 1:59-65; Meares y col., (1990) *J. Cancer* 1990, Suppl. 10:21-26; Izard y col., (1992) *Bioconjugate Chem.* 3:346-350; Nikula y col., (1995) *Nucl. Med. Biol.* 22:387-90; Camera y col., (1993) *Nucl. Med. Biol.* 20:955-62; Kukis y col., (1998) *J. Nucl. Med.* 39:2105-2110; Verel y col., (2003) *J. Nucl. Med.* 44:1663-1670; Camera y col., (1994) *J. Nucl. Med.* 21:640-646; Ruegg y col., (1990) *Cancer Res.* 50:4221-4226; Verel y col., (2003) *J. Nucl. Med.* 44:1663-1670; Lee y col., (2001) *Cancer Res.* 61:4474-4482; Mitchell y col., (2003) *J. Nucl. Med.* 44:1105-1112; Kobayashi y col., (1999) *Bioconjugate Chem.* 10:103-111; Miederer y col., (2004) *J. Nucl. Med.* 45:129-137; DeNardo y col., (1998) *Clinical Cancer Research* 4:2483-90; Blend y col., (2003) *Cancer Biotherapy & Radiopharmaceuticals* 18:355-363; Nikula y col., (1999) *J. Nucl. Med.* 40:166-76; Kobayashi y col., (1998) *J. Nucl. Med.* 39:829-36; Mardirossian y col., (1993) *Nucl. Med. Biol.* 20:65-74; Roselli y col., (1999) *Cancer Biotherapy & Radiopharmaceuticals*, 14:209-20.

(b) Las marcas fluorescentes tales como los quelantes térreos (quelantes europios) poco frecuentes, los tipos fluoresceína, incluyendo FITC, 5-carboxifluoresceína, 6-carboxifluoresceína; los tipos rodamina, que incluyen TAMRA; dansil; lisamina; cianinas; ficoeritrina; Rojo Texas y los análogos de los mismos. Las marcas fluorescentes pueden conjugarse a anticuerpos mediante las técnicas que se describen en, por ejemplo, *Protocols in Immunology*, mencionado anteriormente. Los reactivos de tintes y marcas fluorescentes incluyen aquellos que se encuentran comercialmente disponibles de *Invitrogen/Molecular Probes* (Eugene, OR) y Pierce Biotechnology, Inc. (Rockford, IL).

(c) Se encuentran disponibles o se detallan diversas marcas de sustratos de enzimas (patente de EE.UU. N.º 4275149). Por lo general, la enzima cataliza una alteración química de un sustrato cromogénico que puede medirse mediante diversas técnicas. Por ejemplo, la enzima puede catalizar un cambio de color en un sustrato que puede medirse espectrofotométricamente. Alternativamente, la enzima puede alterar la fluorescencia o quimioluminiscencia de un sustrato. Las técnicas para cuantificar un cambio en fluorescencia se describen anteriormente. El sustrato quimioluminescente se excita electrónicamente mediante una reacción química y entonces puede emitir luz que puede medirse (mediante un quimioluminómetro, por ejemplo) o puede donar energía al aceptor fluorescente. Los ejemplos de marcas enzimáticas incluyen luciferasas (por ejemplo, luciferasa de la luciérnaga y luciferasa bacteriana; patente de EE.UU. N.º 4.737.456), luciferina, 2,3-dihidroftalazinadionas, malato deshidrogenasa, ureasa, peroxidasa tal como la peroxidasa de rábano picante (HRP), fosfatasa alcalina (AP), beta-galactosidasa, glucoamilasa, lisozima, oxidasas sacáridas (como por ejemplo, glucosa oxidasa, galactosa oxidasa y glucosa-6-fosfato deshidrogenasa), oxidasas heterocíclicas (tal como uricasa y xantina oxidasa), lactoperoxidasa, microperoxidasa, y similares. Las técnicas para la conjugación de enzimas con anticuerpos se describen en O'Sullivan y col., (1981) "Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay", en *Methods in Enzym.* (ed J. Langone & H. Van Vunakis), Academic Press, New York, 73:147-166.

[0034] Los ejemplos de combinaciones de sustratos de enzimas incluyen:

(i) Peroxidasa de rábano picante (HRP) con peroxidasa de hidrógeno como un sustrato, donde la peroxidasa de hidrógeno oxida un precursor de tinte (como por ejemplo, el ortofenileno diamina (OPD) o clorhidrato de 3,3',5,5'-tetrametilbenzidina (TMB));

(ii) fosfatasa alcalina (AP) con paranitrofenilfosfato como un sustrato cromogénico; y

(iii) β-D-galactosidasa (β-D-Gal) con un sustrato cromogénico (por ejemplo, p-nitrofenil-β-D-galactosidasa) o un sustrato fluorogénico 4-metilumbeliferil-β-D-galactosidasa.

[0035] Se encuentran disponibles muchas otras combinaciones de sustratos de enzimas para aquellos expertos en la materia. Para obtener una revisión general, véase las patentes de EE.UU. 4275149 y 4318980.

[0036] Una marca puede conjugarse de manera indirecta con una cadena lateral de aminoácidos, una cadena lateral de aminoácidos activada, un anticuerpo diseñado con cisteína, y similares. Por ejemplo, el anticuerpo puede conjugarse con biotina y cualquiera de las tres categorías amplias de marcas mencionadas anteriormente puede conjugarse con avidina o estreptavidina, o viceversa. La biotina se une selectivamente a la estreptavidina y, por ende, la marca puede conjugarse con el anticuerpo de esta manera indirecta. Alternativamente, para lograr la conjugación indirecta de la marca con la variante polipeptídica,

esta última se conjuga con un hapteno pequeño (por ejemplo, digoxina) y uno de los diferentes tipos de marcas mencionadas anteriormente se conjuga con la variante polipeptídica anti-hapteno (por ejemplo, anticuerpo anti-digoxina). Por lo tanto, se puede lograr la conjugación indirecta de la marca con la variante polipeptídica (Hermanson, G. (1996) en *Bioconjugate Techniques*, Academic Press, San Diego)

[0037] El anticuerpo de la presente invención puede emplearse en cualquier método de ensayo conocido tales como ELISA, ensayos de unión competitiva, ensayos tipo "sandwitch" directos e indirectos y ensayos de inmunoprecipitación (Zola, (1987) *Monoclonal Antibodies: A Manual of Techniques*, págs. 147-158, CRC Press, Inc.).

[0038] Una marca de detección puede ser útil para localizar, visualizar y cuantificar una unión o un evento de reconocimiento. Los anticuerpos marcados de la invención pueden detectar receptores en la superficie celular. Otro uso de los anticuerpos marcados de forma detectable es un método de inmunocaptura basado en partículas que comprende la conjugación de una partícula con un anticuerpo fluorescente marcado y la detección de una señal fluorescente sobre la unión de un ligando. Metodologías similares de detección de uniones utilizan el efecto de la resonancia de plasmón superficial (RPS) para medir y detectar las interacciones entre anticuerpos y antígenos.

[0039] Las marcas de detección, como por ejemplo, los tintes fluorescentes y quimioluminescentes (Briggs y col., (1997) "Synthesis of Functionalised Fluorescent Dyes and Their Coupling to Amines and Amino Acids," *J. Chem. Soc.*, Perkin-Trans. 1:1051-1058), proporcionan una señal detectable y por lo general, se aplican en los anticuerpos marcados, preferentemente en aquellos que tienen las siguientes propiedades: (i) el anticuerpo marcado debe producir una señal muy alta con un entorno bajo para que las pequeñas cantidades de anticuerpos puedan detectarse sensiblemente tanto en los ensayos con células como en aquellos sin células; y (ii) el anticuerpo marcado debe ser fotoestable para que la señal fluorescente pueda observarse, controlarse y registrarse sin un fotoblanqueamiento significativo. Para las aplicaciones que implican una unión en la superficie celular del anticuerpo marcado a las membranas o superficies celulares, especialmente células vivas, las marcas preferentemente (iii) deben tener una buena solubilidad en agua para lograr una concentración de conjugado y una sensibilidad de detección eficaces y (iv) no deben ser tóxicas para las células vivas para no alterar los procesos metabólicos normales de las células ni causar la muerte prematura de éstas.

[0040] La cuantificación directa de la intensidad de la fluorescencia celular y la enumeración de los eventos marcados fluorescentemente, como por ejemplo, la unión en la superficie celular de los conjugados de tinte peptídica, pueden realizarse en un sistema (Sistema FMAT® 8100 HTS, Applied Biosystems, Foster City, Calif.) que automatiza la mezcla y lectura de los ensayos no radiactivos con células vivas o partículas (Miraglia, "Homogeneous cell- and bead-based assays for high throughput screening using fluorometric microvolume assay technology", (1999) *J. of Biomolecular Screening* 4:193-

204). Los usos de los anticuerpos marcados también incluyen los ensayos de unión del receptor de superficie celular, los ensayos de inmunocaptura, los ensayos de inmunoabsorción ligado a fluorescencia (FLISA), la escisión de la caspasa (Zheng, "Caspase-3 controls both cytoplasmic and nuclear events associated with Fas-mediated apoptosis in vivo", (1998) *Proc. Natl. Acad. Sci. USA* 95:618-23; US 6372907), la apoptosis (Vermes, "A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V" (1995) *J. Immunol. Methods* 184:39-51) y los ensayos de citotoxicidad. La tecnología de ensayos de microvolumen fluorométrico puede utilizarse para identificar las regulaciones a la alza y a la baja mediante una molécula que actúa sobre la superficie celular (Swartzman, "A homogeneous and multiplexed immunoassay for high-throughput screening using fluorometric microvolume assay technology", (1999) *Anal. Biochem.* 271:143-51).

[0041] Los anticuerpos marcados de la invención son útiles como biomarcadores de diagnóstico por imágenes y sondas que se utilizan en los distintos métodos y técnicas de diagnóstico por imagen biomédica y molecular, tales como: (i) MRI (imágenes obtenidas mediante resonancia magnética); (ii) MicroCT (tomografía computarizada); (iii) SPECT (tomografía computarizada de emisión de fotón único); (iv) PET (tomografía con emisión de positrones) Chen y col., (2004) *Bioconjugate Chem.* 15:41-49; (v) bioluminiscencia; (vi) fluorescencia y (vii) ultrasonido. La inmunoscintigrafía es un procedimiento de diagnóstico por imágenes en el que los anticuerpos marcados con sustancias radiactivas se administran a un animal o a un paciente humano y se obtiene una fotografía de los lugares del cuerpo donde se localizan dichos anticuerpos (patente de EE.UU. N.º 6528624). Los biomarcadores de diagnóstico por imágenes pueden medirse y evaluarse objetivamente como un indicador de procesos biológicos normales, procesos patogénicos o respuestas farmacológicas a una intervención terapéutica. Existen diferentes tipos de biomarcadores: los Tipo 0 son marcadores de la historia natural de una enfermedad y están correlacionados logitudinalmente con índices clínicos conocidos, como por ejemplo, la valoración por MRI de la inflamación sinovial en la artritis reumatoide; los marcadores Tipo I capturan el efecto de una intervención de acuerdo con un mecanismo de acción, inclusive si el mecanismo no está relacionado con el resultado clínico; los marcadores Tipo II funcionan como puntos de valoración alternativos en donde el cambio o la señal del biomarcador pronostica un beneficio clínico para "validar" la respuesta objetiva, como por ejemplo, una erosión ósea que mide la tomografía computarizada en la artritis reumatoide. Por lo tanto, los biomarcadores de diagnóstico por imágenes pueden proporcionar información terapéutica farmacodinámica (FD) sobre: (i) la expresión de una proteína diana, (ii) la unión de un agente terapéutico a la proteína diana, es decir, la selectividad, y (iii) los datos de eliminación y semivida farmacocinética. Las ventajas de los biomarcadores de imágenes *in vivo* con respecto a los biomarcadores basados en el laboratorio incluyen: tratamiento no invasivo, cuantificación, evaluación de todo el cuerpo, evaluaciones y dosis repetitivas, es decir, muchos momentos específicos, y transferencia potencial de efectos preclínicos (en animales

pequeños) a resultados clínicos (en humanos). Para algunas aplicaciones, la bioimagen sustituye o minimiza la cantidad de experimentos realizados en animales en estudios preclínicos.

[0042] Los métodos de marcado con péptidos son bien conocidos. Véase Haugland, 2003, *Molecular Probes Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc.; Brinkley, 1992, *Bioconjugate Chem.* 3:2; Garman, (1997) *Non-Radioactive Labelling: A Practical Approach, Academic Press*, London; Means (1990) *Bioconjugate Chem.* 1:2; Glazer y col., (1975) *Chemical Modification of Proteins*. Laboratory Techniques in Biochemistry and Molecular Biology (T. S. Work y E. Work, Eds.) American Elsevier Publishing Co., New York; Lundblad, R. L. y Noyes, C. M. (1984) *Chemical Reagents for Protein Modification*, Vols. I y II, CRC Press, New York; Pfleiderer, G. (1985) "Chemical Modification of Proteins", *Modern Methods in Protein Chemistry*, H. Tschesche, Ed., Walter DeGryter, Berlin y New York; y Wong (1991) *Chemistry of Protein Conjugation and Cross-linking*, CRC Press, Boca Raton, Fla.); De Leon-Rodriguez y col., (2004) *Chem.Eur. J.* 10:1149-1155; Lewis y col., (2001) *Bioconjugate Chem.* 12:320-324; Li y col., (2002) *Bioconjugate Chem.* 13:110-115; Mier y col., (2005) *Bioconjugate Chem.* 16:240-237.

[0043] Los péptidos y las proteínas marcados con dos grupos, un indicador fluorescente y uno de absorción lo suficientemente cerca entre sí experimentan la transferencia resonante de energía de fluorescencia (FRET). Por lo general, los grupos de indicadores son tintes fluorescentes que se excitan mediante la luz a una longitud de onda determinada y transfieren la energía a un grupo de aceptores o absorbentes con el cambio Stokes adecuado para producir la emisión con un brillo máximo. Los tintes fluorescentes incluyen moléculas con una aromaticidad extendida tal como la fluoresceína y la rodamina y sus derivados. La fracción absorbente en un péptido intacto puede absorber parcial o completamente el indicador fluorescente. Cuando la peptidasa o la protasa producen la escisión de un péptido, puede medirse un aumento detectable en la fluorescencia (Knight, C. (1995) "Fluorimetric Assays of Proteolytic Enzymes", *Methods in Enzymology*, Academic Press, 248:18-34).

[0044] Los anticuerpos marcados de la invención también pueden utilizarse como un agente para la purificación por afinidad. En este proceso, el anticuerpo marcado se inmoviliza en un soporte sólido, como una resina Sephadex o papel de filtro, utilizando métodos conocidos en la materia. El anticuerpo inmovilizado entra en contacto con una muestra que contiene el antígeno que deberá purificarse y, a continuación, el soporte se lava con un solvente adecuado que eliminará sustancialmente todo el material de la muestra, excepto el antígeno que se purificará y que está unido a la variante polipeptídica inmovilizada. Finalmente, el soporte se lava con otro solvente adecuado tal como el tampón glicina, pH 5,0, que liberará el antígeno de la variante polipeptídica.

[0045] Los reactivos de marcación tienen generalmente una funcionalidad reactiva que puede reaccionar (i) directamente con un tiol de cisteína de un anticuerpo diseñado con cisteína para formar un

anticuerpo marcado, (ii) con un reactivo enlazador para formar un enlazador marcado intermedio o (iii) con un anticuerpo enlazador para formar un anticuerpo marcado. La funcionalidad reactiva de los reactivos de marcación incluye: maleimido, haloacetil, éster succinimidil iodoacetamida (por ejemplo, NHS, N-hidroxisuccinimida), isotiocianato, cloruro sulfonil, 2,6-diclorotriazinil, éster pentafluorofenil y fosforamidita, a pesar de que también pueden utilizarse otros grupos funcionales.

[0046] Un grupo funcional reactivo ejemplar es el éster N-hidroxisuccinimidil (NHS) de un grupo carboxilo que sustituye una marca detectable, por ejemplo, una biotina o un tinte fluorescente. El éster NHS de la marca puede preformarse, aislarse, purificarse o caracterizarse, o todos los anteriores, o bien puede formarse in situ y reaccionar con un grupo nucleofílico de un anticuerpo. Por lo general, la forma carboxilo de la marca se activa cuando reacciona con alguna combinación de un reactivo de carbodiimida, como por ejemplo, diciclohexilcarbodiimida, diisopropilcarbodiimida, o un reactivo de uronio, como por ejemplo, TSTU (O-(N-Succinimidil)-N,N,N',N'-tetrametiluronio tetrafluoroborato, HBTU (O-benzotriazol-1-yl)-N,N,N',N'-tetrametiluronio hexafluorofosfato) o HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetrametiluronio hexafluorofosfato), un activador tal como 1-hidroxibenzotriazol (HOBt) y N-hidroxisuccinimida para proporcionar el éster NHS de la marca. En algunos casos, la marca y el anticuerpo deben acoplarse mediante la activación de la marca en el lugar y reaccionar con el anticuerpo para formar el conjugado de anticuerpo y marca en un paso. Otros reactivos de activación y acoplamiento incluyen TBTU (2-(1H-benzotriazol-1-il)-1-1,3,3-tetrametiluronio hexafluorofosfato), TFFH (N,N',N",N'"-tetrametiluronio 2-fluoro-hexafluorofosfato), PyBOP (benzotriazol-1-il-oxi-tris-pirrolidino-fosfonio hexafluorofosfato, EEDQ (2-etoxi-1-etoxicarbonil-1,2-dihidroquinolina), DCC (diciclohexilcarbodiimida); DIPCDI (diisopropilcarbodiimida), MSNT (1-(mesitileno-2-sulfonil)-3-nitro-1H-1,2,4-triazolo y arilo sulfonil halidos, como ejemplo, cloruro de triisopropilbenzensulfonil.

Compuestos de péptidos-Fab de unión a la albúmina de la invención:

[0047] En un aspecto, el anticuerpo de la invención se fusiona con una proteína de unión a la albúmina. La unión a proteínas plasmáticas puede ser una manera eficaz de mejorar las propiedades farmacocinéticas de las moléculas de vida corta. La albúmina es la proteína que se encuentra en mayor abundancia en el plasma. Los péptidos de unión a la albúmina sérica (ABP) pueden alterar la farmacodinámica de las proteínas fusionadas al dominio activo, incluyendo la alteración de la absorción, penetración y difusión de tejido. Estos parámetros farmacodinámicos pueden modularse mediante la selección específica de la secuencia adecuada del péptido de unión a la albúmina sérica (US 20040001827). Se identificaron una serie de péptidos de unión a la albúmina mediante la detección de representación en imágenes de fagos (Dennis y col., (2002) *"Albumin Binding As A General Strategy for Improving The Pharmacokinetics Of Proteins"* J Biol Chem. 277:35035-35043; documento WO 01/45746). Los compuestos de la invención incluyen secuencias de péptidos de unión a la albúmina (ABP) que se indican

en: (i) Dennis y col., (2002) *J Biol Chem.* 277:35035-35043 en las Tablas III y IV, página 35038; (ii) US 20040001827 en [0076] identificadores de secuencia n.º: 9-22; y (iii) en el documento WO 01/45746 en las páginas 12-13, los cuales se incorporan en la presente memoria descriptiva a modo de referencia. Los Fabs de unión a la albúmina (ABP) están diseñados mediante la fusión de un péptido de unión a la albúmina y, por ejemplo, el extremo C-terminal de la cadena pesada de Fab con un coeficiente estequiométrico de 1:1 (1 ABP / 1 Fab). Se demostró que la asociación de estos ABP-Fabs con la albúmina incrementó la semivida del anticuerpo más de 25 veces en conejos y ratones. Por lo tanto, los residuos Cis reactivos mencionados anteriormente pueden introducirse en estos ABP-Fabs y utilizarse para la conjugación específica de un punto con fármacos citotóxicos seguidos de estudios *in vivo* en animales.

[0048] Las secuencias de péptidos de unión a la albúmina ejemplares incluyen, entre otras, las secuencias de animoácidos que se enumeran en los identificadores de secuencia n.º:80-84:

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	Identificador de secuencia n.º:80
QRLMEDICLPRWGCLWEDDF	Identificador de secuencia n.º:81
QRLIEDICLPRWGCLWEDDF	Identificador de secuencia n.º:82
RLIEDICLPRWGCLWEDD	Identificador de secuencia n.º:83
DICLPRWGCLW	Identificador de secuencia n.º:84

Conjugados de anticuerpos con fármacos

[0049] En otro aspecto, la invención proporciona inmunoconjugados, conjugados de anticuerpos con fármacos (ADC), que comprenden un anticuerpo conjugado con un agente citotóxico, como por ejemplo un agente quimioterapéutico, un fármaco, un agente inhibidor del crecimiento, una toxina (por ejemplo, una toxina enzimáticamente activa de origen bacteriano, fúngico, animal o vegetal, o fragmentos de la misma), o un isótopo radioactivo (es decir, un radioconjugado). En otro aspecto, la invención también ofrece métodos de uso de los inmunoconjugados. En un aspecto, un inmunoconjugado comprende cualquiera de los anticuerpos anti-STEAP-1 mencionados anteriormente unidos covalentemente a un agente citotóxico o detectable.

[0050] El uso de anticuerpos conjugados con fármacos para la administración local de agentes citotóxicos o citostáticos, es decir, los fármacos que se utilizan para matar o inhibir células tumorales en el tratamiento del cáncer (Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz y Springer (1997) *Adv. Drg Del. Rev.* 26: 151 -172; patente de EE. UU. n.º 4.975.278), permite la administración de la fracción de fármacos a tumores y una acumulación intracelular en éstos, donde la administración sistemática de estos agentes farmacológicos no conjugados puede resultar en niveles inaceptables de toxicidad tanto para las células normales como para las células tumorales que se intentan eliminar (Baldwin y col., (1986) *Lancet* págs. (Mar. 15, 1986):603-05; Thorpe, (1985) "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," en *Monoclonal Antibodies '84: Biological And*

Clinical Applications, A. Pinchera y col. (eds), págs. 475-506). Se busca entonces la máxima eficacia con la mínima toxicidad. Tanto los anticuerpos monoclonales como los policlonales se han descrito como útiles en estas estrategias (Rowland y col., (1986) *Cancer Immunol. Immunother.*, 21:183 (-87). Los fármacos empleados en estos métodos incluyen a la daunomicina, la doxorrubicina, el metotrexato y la vindesina (Rowland y col., (1986) mencionado anteriormente). Entre las toxinas empleadas en los conjugados de anticuerpos con toxinas se encuentran toxinas bacterianas como la toxina de la difteria, toxinas vegetales como la ricina, toxinas de molécula pequeña como la geldanamicina (Mandler y col., (2000) *Jour. of the Nat. Cancer Inst.* 92(19):1573-1581; Mandler y col., (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler y col., (2002) *Bioconjugate Chem.* 13:786-791), maitansinoides (EP 1391213; Liu y col., (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623) y caliqueamicina (Lode y col. (1998) *Cancer Res.* 58:2928; Hinman y col., (1993) *Cancer Res.* 53:3336-3342). Las toxinas pueden producir sus efectos citotóxicos y citostáticos mediante mecanismos que incluyen la unión de tubulina, la unión de ADN o la inhibición de la topoisomerasa. Algunos fármacos citotóxicos tienden a ser inactivos o menos activos cuando se conjugan con grandes anticuerpos o ligandos receptores de proteínas.

[0051] ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) es un anticuerpo conjugado con un radioisótopo compuesto de un anticuerpo monoclonal kappa IgG1 murino dirigido contra el antígeno CD20 que se encuentra en la superficie de los linfocitos B cancerígenos y normales, y contra el radioisótopo ^{111}In o ^{90}Y unido por un quelante enlazador de tiourea (Wiseman y col., (2000) *Eur. Jour. Nucl. Med.* 27(7):766-77; Wiseman y col., (2002) *Blood* 99(12):4336-42; Witzig y col., (2002) *J. Clin. Oncol.* 20(10):2453-63; Witzig y col., (2002) *J. Clin. Oncol.* 20(15):3262-69). A pesar de que el ZEVALIN presenta actividad contra las células B del linfoma no Hodgkin (LNH), su administración provoca citopenias graves y prolongadas en la mayoría de los pacientes. MYLOTARG™ (Gentuzumab ozogamicina, Wyeth Pharmaceuticals), un conjugado de anticuerpos y fármacos compuesto de un anticuerpo huCD33 enlazado a caliqueamicina, se aprobó en 2000 para el tratamiento de leucemia mieloide aguda por inyección (*Drugs of the Future* (2000) 25(7): 686; Patentes de EE.UU. n.º 4.970.198; 5.079.233; 5.585.089; 5.606.040; 5.693.762; 5.739.116; 5.767.285; 5.773.001). Cantuzumab mertansina (Immunogen, Inc.), un conjugado de un anticuerpo con fármacos compuesto por el anticuerpo huC242 enlazado a la fracción de fármacos de los maitansinoides por medio de un enlazador de disulfuro SPP, DM1, está bien avanzado en ensayos clínicos de Fase II para el tratamiento de cánceres que expresan CanAg, como los de colon, páncreas, gástricos y otros. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), un anticuerpo conjugado con un fármaco, compuesto por el anticuerpo monoclonal del antígeno de membrana antiprostático (PSMA) enlazado al grupo de los maitansinoides, DM1, está en fase de desarrollo para el tratamiento potencial de los tumores de próstata. Los péptidos auriestatinos, la auristatina E (AE) y la monometilauristatina (MMAE), análogos sintéticos de la dolastatina, se conjugaron a anticuerpos monoclonales quiméricos cBR96 (específicos para Lewis Y en carcinomas) y cAC10 (específicos para

CD30 en tumores hematológicos) (Doronina y col., (2003) *Nature Biotechnology* 21(7):778-784) y se encuentran en desarrollo terapéutico.

[0052] Los agentes quimioterapéuticos útiles en la generación de inmunoconjugados se describen en la presente memoria descriptiva. Las toxinas enzimáticamente activas y los fragmentos de las mismas que se pueden utilizar incluyen la cadena de difteria A, los fragmentos activos sin unión de la toxina de la difteria, la cadena de exotoxina A (de *Pseudomonas aeruginosa*), la cadena de ricina A, la cadena de abrina A, la cadena de modecina A, la alfa-sarcina, las proteínas de *Aleurites fordii*, las proteínas de diantina, las proteínas de *Phytolaca americana* (PAPI, PAPII y PAP-S), el inhibidor de la Momordica charantia, la curcina, la crotina, el inhibidor de la Saponaria officinalis, la gelonina, la mitogelina, la restrictocina, la fenomicina, la enomicina y el tricotecenos. Véase, por ejemplo, el documento WO 93/21232, publicado el 28 de octubre de 1993. Se dispone de diversos radionúclidos para la producción de anticuerpos radioconjugados. Entre los ejemplos se incluyen ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y y ^{186}Re. Los conjugados del anticuerpo y el agente citotóxico se pueden realizar utilizando una variedad de agentes de unión a proteína bifuncionales, como por ejemplo N-succinimidil-3-(2-piridilditio) propionato (SPDP), iminotiolano (IT), derivados bifuncionales de imidoésteres (como el dimetiladipimidato HCl), ésteres activos (por ejemplo, disuccinimidil suberato), aldehídos (por ejemplo, glutaraldehídos), compuestos bis-azido (como bis (p-azidobenzoil) hexanodiamina), derivados de bis-diazonio (como bis-(p-diazoniobenzoil)-etilenodiamina), diisocianatos (por ejemplo, 2,6-diisocianato de tolueno) y compuestos bis-activos de fluoruro (como 1,5-difluoruro-2,4-dinitrobenceno). For ejemplo, una inmunotoxina de ricina puede prepararse como se describe en Vitetta y col., (1987) *Science*, 238:1098. El ácido triaminopentaacético 1-isotiocianatobencil-3-metildietileno marcado con carbono 14 (MX-DTPA) es un ejemplo de agente quelante para la conjugación de un radionucleótido a un anticuerpo (documento W0 94/11026).

[0053] También se contemplan en la presente memoria descriptiva los conjugados de un anticuerpo y una o más toxinas de molécula pequeña, como una calicheamicina, maitansinoides, dolastatinas, auristatinas, un tricoteceno y un CC1065, y los derivados de estas toxinas que tengan actividad tóxica.

Maitansina y maitansinoides

[0054] En algunas realizaciones, el inmunoconjugado comprende un anticuerpo de la invención (longitud completa o fragmentos) conjugado con una o más moléculas de maitansinoides.

[0055] Los maitansinoides son inhibidores mitóticos que actúan inhibiendo la polimerización de la tubulina. La maitansina se aisló por primera vez a partir del arbusto de África oriental *Maytenus serrata* (patente de EE.UU. N.º 3896111). Posteriormente, se descubrió que determinados microbios también producían maitansinoides, como el maitansinol y los ésteres de C-3 amitansinol (patente de EE.UU. N.º 4.151.042). El maitansinol sintético y sus derivados y análogos se describen, por ejemplo, en las

patentes de EE.UU. N.º 4.137.230; 4.248.870; 4.256.746; 4.260.608; 4.265.814; 4.294.757; 4.307.016; 4.308.268; 4.308.269; 4.309.428; 4.313.946; 4.315.929; 4.317.821; 4.322.348; 4.331.598; 4.361.650; 4.364.866; 4.424.219; 4.450.254; 4.362.663 y 4.371.533.

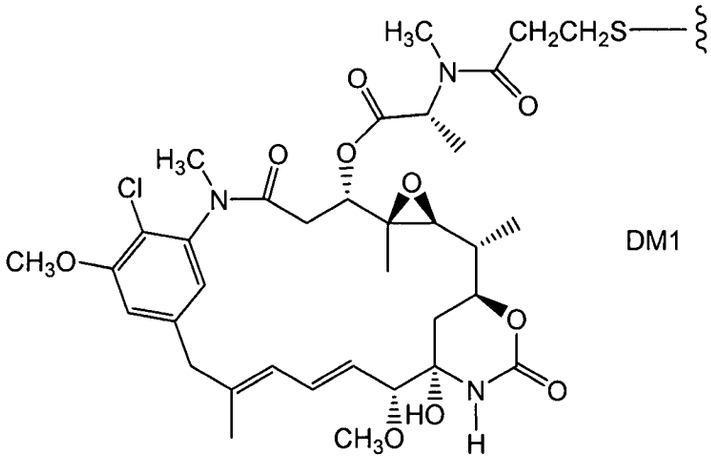
[0056] Los grupos de fármacos maitansinoides son grupos de fármacos que resultan atractivos para la elaboración de anticuerpos conjugados con fármacos por sus siguientes características: (i) son relativamente accesibles en lo que se refiere a su preparación mediante fermentación, modificación química o derivación de productos de fermentación, (ii) son fáciles de someter a derivación, con grupos funcionales adecuados para conjugación a través de los enlazadores no disulfuro con anticuerpos, (iii) tienen estabilidad en plasma y (iv) resultan efectivos frente a distintas líneas celulares tumorales.

[0057] Los compuestos de maitansina aptos para uso como grupos de fármacos maitansinoides son bien conocidos en la materia y se pueden aislar de fuentes naturales según métodos conocidos o se pueden producir mediante técnicas de ingeniería genética (véase Yu y col., (2002) *PNAS* 99:7968-7973), o utilizando maitansinol y sus análogos preparados de forma sintética mediante métodos conocidos.

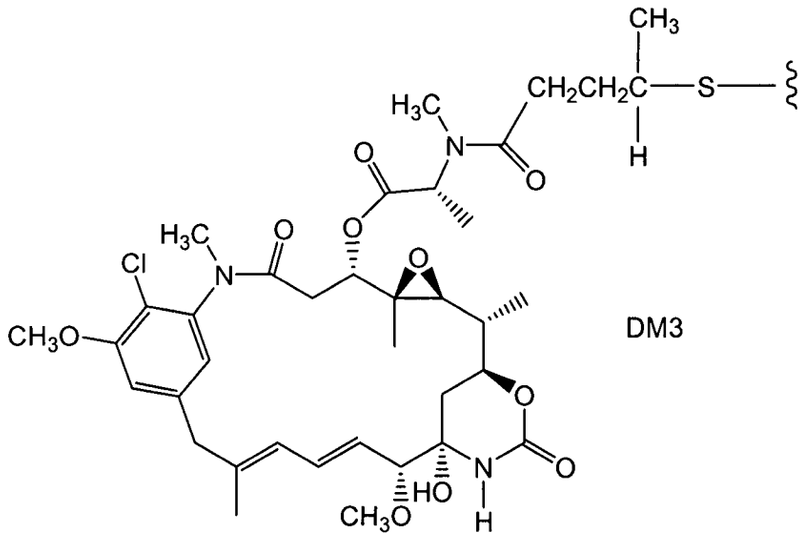
[0058] Entre los grupos ejemplares de fármacos maitansinoides están los que tienen un anillo aromático modificado, como por ejemplo: C-19-dicloro (patente de EE.UU. N.º 4256746) (preparado por reducción de ansamitocina P2 mediante hidruro de litio-aluminio); C-20-hidroxi (o C-20-dimetil) +/-C-19-dicloro (patentes de EE.UU. N.º 4361650 y 4307016) (preparado mediante desmetilación utilizando *Streptomyces* o *Actinomyces* o declorinación con LAH); y C-20-dimetoxi, C-20-aciloxi (-OCOR), +/-dicloro (patente de EE.UU. N.º 4.294.757) (preparado mediante acilación usando cloruros de acilo) y aquellos que tienen modificaciones en otras posiciones.

[0059] Entre los grupos ejemplares de fármacos maitansinoides también están los que tienen modificaciones como por ejemplo: C-9-SH (patente de EE.UU. N.º 4424219) (preparado mediante reacción de maitansinol con H_2S o P_2S_5); C-14-alcoximetil(dimetoxi/CH_2 OR)(patente de EE.UU. N.º 4331598); C-14-hidroximetil o aciloximetil (CH_2OH o CH_2OAc) (patente de EE.UU. N.º 4450254) (preparado a partir de Nocardia); C-15-hidroxi/aciloxi (patente de EE.UU. N.º 4364866) (preparado mediante la conversión de maitansinol por Streptomyces); C-15-metoxi (patentes de EE.UU. N.º 4313946 y 4315929) (aislado a partir de Trewia nudiflora); C-18-N-dimetil (patentes de EE.UU. N.º 4362663 y 4322348) (preparado por desmetilación de maitansinol por Streptomyces); y 4,5-deoxi (US 4371533) (preparado por la reducción de maitansinol mediante tricloruro de titanio/LAH).

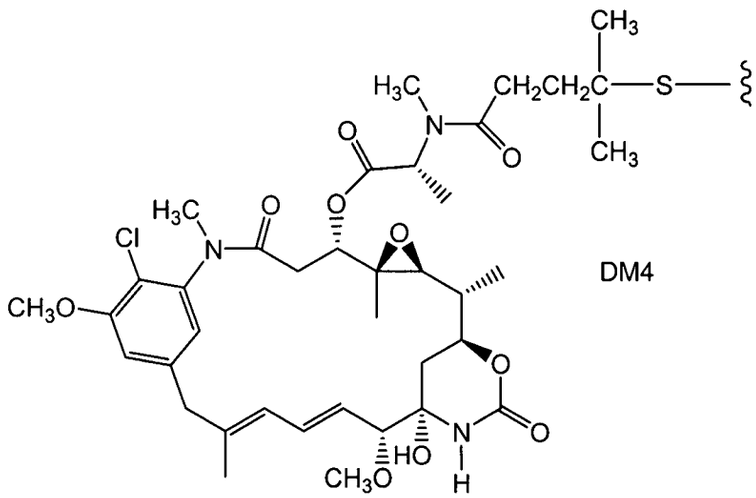
[0060] A continuación se citan algunos ejemplos de formas de realización ejemplares de grupos de fármacos maitansinoides: DM1; DM3; y DM4, con las estructuras:



DM1



DM3

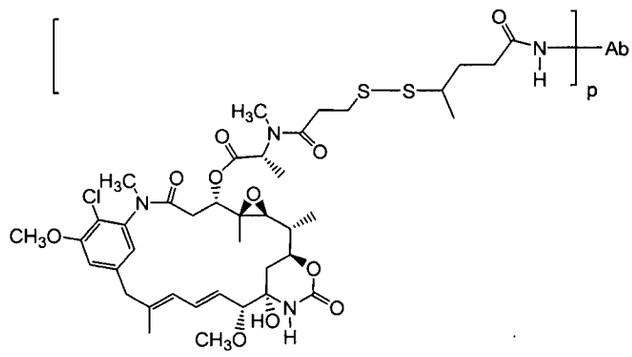


DM4

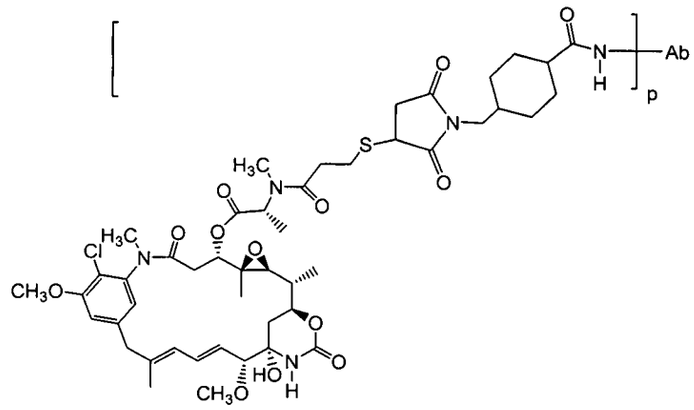
donde la línea ondulada indica el enlace covalente del átomo de sulfuro del fármaco a un enlazador (L) de un conjugado de anticuerpo con fármaco. Se ha informado sobre el HERCEPTIN® (trastuzumab)

enlazado por SMCC a DM (documento WO 2005/037992, que se incorpora en la presente memoria descriptiva a modo de referencia). Un conjugado de anticuerpo con fármacos de la presente invención se puede preparar de acuerdo con los procedimientos divulgados en esta memoria descriptiva.

[0061] Otros conjugados ejemplares de anticuerpo con fármacos maitansinoides tienen las siguientes estructuras y abreviaturas (donde Ab es el anticuerpo y p es 1 a aproximadamente 8):

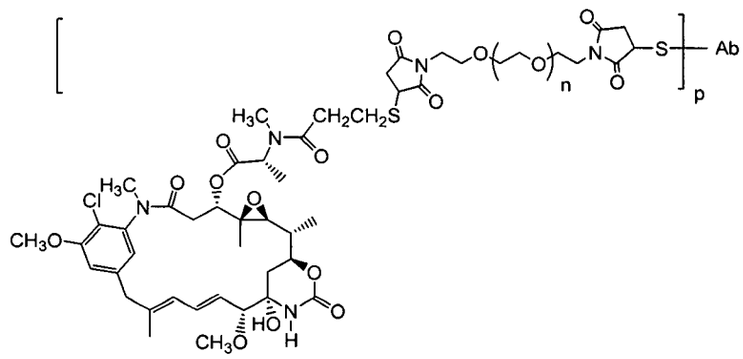


Ab -SPP-DM1



Ab-SMCC-DM1

[0062] Los conjugados ejemplares de un anticuerpo con fármacos donde DM1 se enlaza a través de un enlazador BMPEO a un grupo tiol del anticuerpo tienen la estructura y abreviatura:



donde Ab es el anticuerpo; n es 0, 1 ó 2; y p es 1, 2, 3 ó 4.

[0063] Los inmunoconjugados que contienen maitansinoides, los métodos para elaborarlos y su uso terapéutico se describen, por ejemplo, en las patentes de EE.UU N.º 5.208.020 y 5.416.064, 6.441.163 y en la patente Europea EP 0 425 235 B1, y los descubrimientos de las mismas se incorporan expresamente a la presente memoria descriptiva a modo de referencia. Liu y col., *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996) describe los inmunoconjugados que comprenden un maitansinoide denominado DM1 enlazado al anticuerpo monoclonal C242 dirigido contra el cáncer colorrectal humano. Se descubrió que el conjugado era altamente citotóxico para las células del cáncer de colon en cultivo y demostró actividad antitumoral en ensayos de crecimiento tumoral *in vivo*. Chari y col., *Cancer Research* 52:127-131 (1992) describen inmunoconjugados en los que un maitansinoide se conjugaba por medio de un enlazado de disulfuro al anticuerpo murino A7 que se une a un antígeno en las líneas celulares de cáncer de colon humanas, o a otro anticuerpo monoclonal murino TA.1 que se une al oncogen HER-2/neu. La citotoxicidad del conjugado TA.1-maitansinoide se probó *in vitro* en la línea celular de cáncer de mama SK-BR-3, que expresa 3 x 10^5 antígenos de superficie HER-2 por célula. El conjugado de fármaco consiguió un alto grado de citotoxicidad similar al fármaco maitansinoide libre, que podría ser mayor si se aumentase el número de moléculas maitansinoides por cada molécula de anticuerpo. El conjugado A7-maitansinoide mostró una citotoxicidad sistémica baja en ratones.

[0064] Los conjugados de un anticuerpo con un maitansinoide anti-STEAP-1 se preparan mediante enlace químico de un anticuerpo a una molécula de maitansinoide sin hacer que disminuya significativamente la actividad biológica de ambos elementos del conjugado. Véase, por ejemplo, la patente de EE.UU. N.º 5.208.020 (cuyos descubrimientos se incorporan expresamente a la presente memoria descriptiva a modo de referencia). Una media de 3-4 moléculas maitansinoides conjugadas por anticuerpo ha mostrado eficacia en la mejora de la citotoxicidad de las células diana sin afectar negativamente a la función o la solubilidad del anticuerpo, aunque cabría esperar incluso que una molécula toxina/anticuerpo mejore la citotoxicidad en relación con el uso del anticuerpo desnudo. Los maitansinoides son bien conocidos en la materia y pueden sintetizarse mediante técnicas conocidas o aislarse de fuentes naturales. En la patente de EE.UU. N.º 5.208.020 y en otras patentes y publicaciones no patentadas a las que anteriormente se ha hecho referencia, por ejemplo, se describen maitansinoides adecuados. Los maitansinoides preferidos son el maitansinol y análogos modificados del maitansinol, en el anillo aromático o en otras posiciones de la molécula de maitansinol, como distintos ésteres del maitansinol.

[0065] Se conocen muchos grupos de enlace en la materia para producir conjugados de anticuerpos con maitansinoides, incluidos, por ejemplo, los descritos en las patentes de EE.UU. N.º 5.208.020, 6.441.163 o patente europea 0 425 235 B1, Chari y col., *Cancer Research* 52:127-131 (1992), y

de la patente de EE.UU. N.º 2005/0169933 A1, y lo descrito en los mismos se incorpora expresamente en la presente memoria descriptiva a modo de referencia. Los conjugados de anticuerpos con maitansinoide que incluyen el componente enlazador SMCC pueden prepararse como se indica en la solicitud de patente de EE.UU. N.º 11/141344, presentada el 31 de mayo de 2005, "*Antibody Drug Conjugates and Methods*". Los grupos enlazadores comprenden grupos disulfuro, grupos de tioéteres, grupos lábiles al ácido, grupos fotolábiles, grupos lábiles a la peptidasa o grupos lábiles a la esterasa, como se revela en las patentes mencionadas anteriormente. En la presente memoria descriptiva se dan ejemplos y se describen grupos enlazadores adicionales.

[0066] Los conjugados del anticuerpo y el maitansinoide se pueden realizar utilizando una variedad de agentes de unión a proteína bifuncionales, como por ejemplo N-succinimidil-3-(2-piridilditio) propionato (SPDP), succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato (SMCC), iminotiolano (IT), derivados bifuncionales de imidoésteres (como el dimetiladipimidato HCl), ésteres activos (por ejemplo, disuccinimidil suberato), aldehídos (por ejemplo, glutaraldehídos), compuestos bis-azido (como bis (p-azidobenzoil) hexanodiamina), derivados de bis-diazonio (como bis-(p-diazoniobenzoil)-etilenodiamina), diisocianatos (por ejemplo, 2,6-diisocianato de tolueno) y compuestos bis-activos de fluoruro (como 1,5-difluoruro-2,4-dinitrobenceno). Los agentes de unión que se prefieren sobre todo son N-sucinimidil-3-(2-piridilditio)propionato (SPDP) (Carlsson et al., *Biochem. J.* 173:723-737 [1978]) y N-sucinimidil-4-(2-piridilditio)pentanoato (SPP) para proporcionar un enlace de disulfido.

[0067] El enlazador se puede unir a la molécula de maitansinoide en varias posiciones, dependiendo del tipo de enlace. Por ejemplo, un enlace con éster se puede formar mediante reacción con un grupo hidroxilo que utilice técnicas de unión convencionales. La reacción puede producirse en la posición C-3 que tiene un grupo hidroxilo, la posición C-14 modificada con hidroximetilo, la posición C-15 modificada con un grupo hidroxilo y la posición C-20 que tiene un grupo hidroxilo. En una forma de realización preferente, el enlace se forma en la posición 3 del maitansinol o un análogo del maitansinol.

[0068] En una forma de realización, cualquiera de los anticuerpos de la invención (longitud completa o fragmentos) se conjuga con una o más moléculas de maitansinoides. En un forma de realización del inmunoconjugado, el agente citotóxico D es un maitansinoide DM 1. En una forma de realización del inmunoconjugado, el ligando se selecciona del grupo compuesto por SPDP, SMCC, IT, SPDP, y SPP.

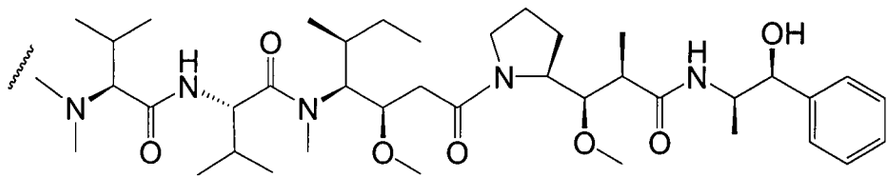
Auristatinas y dolostatinas

[0069] En algunas formas de realización, el inmunoconjugado comprende un anticuerpo de la invención conjugado con dolastatinas o análogos peptídicos a la dolastatina y derivados, las auristatinas (patentes de EE.UU. N. º 5.635.483; 5.780.588). Se ha establecido que las dolastatinas y las auristatinas interfieren con la dinámica de microtúbulos, la hidrólisis GTP y la división nuclear y celular (Woyke y col.,

(2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) y tienen actividad anticancerígena (patente de EE.UU. N.º 5.663.149) y antifúngica (Pettit y col., (1998) *Antimicrob. Agents Chemother.* 42:2961-2965). La fracción del fármaco auristatina o dolastatina puede estar unida al anticuerpo a través de los extremos terminales N (amino) o C (carboxil) de la fracción péptidico del fármaco (documento WO 02/088172).

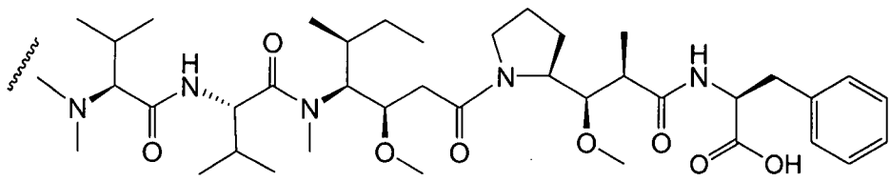
[0070] Entre las formas de realización ejemplares de auristatina están los grupos de fármacos DE y DF de monometilauristatina enlazados al extremo N-terminal, descritas en Senter y col., "*Proceedings of the American Association for Cancer Research*", Volume 45, Abstract Number 623, presentada el 28 de marzo de 2004, cuyo contenido se incorpora expresamente en su totalidad a modo de referencia.

[0071] Una forma ejemplar de realización de auristatina es MMAE (donde la línea ondulada indica el enlace covalente a un enlazador (L) de un conjugado de anticuerpo con fármaco).



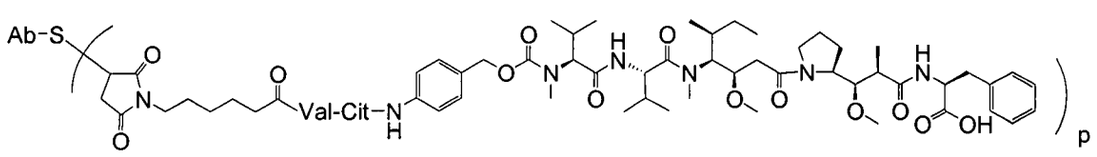
MMAE

[0072] Otra forma ejemplar de realización de auristiatina es MMAF, donde la línea ondulada indica el enlace covalente a un enlazador (L) de un conjugado de anticuerpo con fármaco (patente de EE.UU. N.º 2005/0238649):

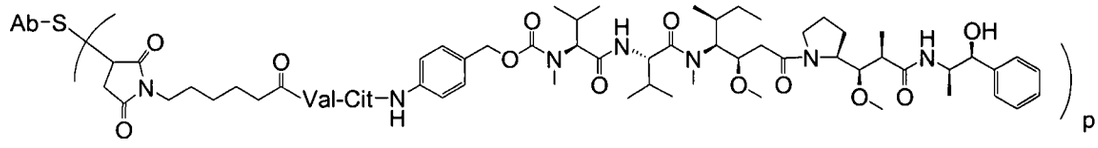


MMAF

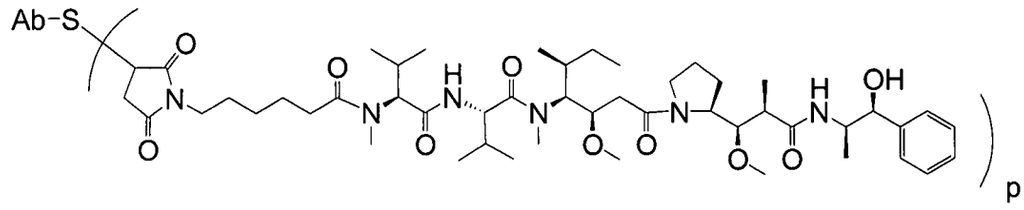
[0073] Otros ejemplos de realizaciones ejemplares que comprenden MMAE o MMAF y varios componentes enlazadores (descritos en la presente memoria en mayor detalle) tienen las siguientes estructuras y abreviaturas (donde Ab significa anticuerpo y p es 1 a aproximadamente 8):



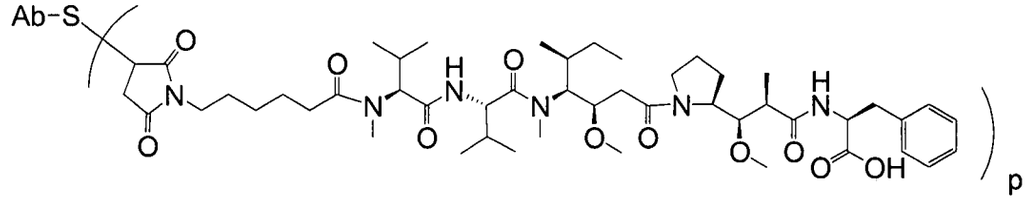
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

[0074] Por lo general, los grupos de fármacos basados en péptidos pueden prepararse formando un enlace peptídico entre dos o más aminoácidos y/o fragmentos de péptidos. Estos enlaces peptídicos pueden prepararse, por ejemplo, de acuerdo con el método de síntesis de fase líquida (véase E. Schröder y K. Lübke, "*The Peptides*", volumen 1, págs. 76-136, 1965, Academic Press) que es bien conocido en el campo de la química de péptidos. Los grupos de fármacos auristatina/dolastatina pueden prepararse de acuerdo con los siguientes métodos: patente de EE.UU. N.º 5.635.483; patente de EE.UU. N.º 5.780.588; Pettit y col., (1989) *J. Am. Chem. Soc.* 111:5463-5465; Pettit y col., (1998) *Anti-cáncer Drug Design* 13:243-277; Pettit, G.R., y col., *Synthesis*, 1996, 719-725; Pettit y col., (1996) *J. Chem. Soc. Perkin Trans.* 1 5:859-863; y Doronina (2003) *Nat. Biotechnol.* 21(7):778-784.

Caliqueamicina

[0075] En otras realizaciones, el inmunoconjugado comprende un anticuerpo de la invención conjugado con una o más moléculas de calicheamicina. La familia de antibióticos de la calicheamicina es capaz de producir la ruptura del ADN de hebra doble en concentraciones por debajo de picomolar. Para la preparación de conjugados de la familia de la caliqueamicina, véanse las patentes de EE.UU. N.º 5.712.374, 5.714.586, 5.739.116, 5.767.285, 5.770.701, 5.770.710, 5.773.001 y 5.877.296 (todas para American Cyanamid Company). Entre análogos estructurales de la caliqueamicina que se pueden utilizar se incluyen,

entre otros, γ_1^I, α_2^I, α_3^I, N-acetil-γ_1^I, PSAG y θ^I_1 (Hinman y col., *Cancer Research* 53:3336-3342 (1993), Lode y col., *Cancer Research* 58:2925-2928 (1998) y las patentes de EE.UU. anteriormente mencionadas para American Cyanamid). Otro fármaco antitumoral con el que es posible conjugar el anticuerpo es QFA, que es un atifolato. Tanto la caliqueamicina como el QFA tienen puntos intracelulares de acción y no atraviesan fácilmente la membrana plasmática. Por ello, la absorción celular de estos agentes a través de la internalización mediada por el anticuerpo potencia enormemente sus efectos citotóxicos.

Otros agentes citotóxicos

[0076] Otros agentes antitumorales que se pueden conjugar con los anticuerpos de la invención incluyen BCNU, estreptozoicina, vincristina y 5-fluorouracilo, la familia de agentes conocidos colectivamente como complejo LL-E33288 descrito en las patentes de EE.UU. N.º 5.053.394, 5.770.710, así como las esperamicinas (patente de EE.UU. N.º 5.877.296).

[0077] Las toxinas enzimáticamente activas y los fragmentos de las mismas que se pueden utilizar incluyen cadena de difteria A, fragmentos activos sin unión de la toxina de la difteria, cadena de exotoxina A (de *Pseudomonas aeruginosa*), cadena de ricina A, cadena de abrina A, cadena de modecina A, alfa-sarcina, proteínas de *Aleurites fordii*, proteínas de diantina, proteínas de *Phytolaca americana* (PAPI, PAPII y PAP-S), inhibidor de la Momordica charantia, curcina, crotina, inhibidor de la Saponaria officinalis, gelonina, mitogelina, restrictocina, fenomicina, enomicina y el tricotecenos. Véase, por ejemplo, el documento WO 93/21232 publicado el 28 de octubre de 1993.

[0078] Esta invención también contempla un inmunoconjugado formado entre un anticuerpo y un compuesto con actividad nucleolítica (por ejemplo, una ribonucleasa o una endonucleasa de ADN como por ejemplo la desoxirribonucleasa; ADNasa).

[0079] Para la destrucción selectiva del tumor, el anticuerpo puede comprender un átomo altamente radioactivo. Se dispone de una variedad de isótopos radiactivos para la producción de anticuerpos radioconjugados. Entre los ejemplos se incluyen At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} e isótopos radiactivos de Lu. Cuando el conjugado se utiliza para la detección, puede comprender un átomo radioactivo para gammagrafía, por ejemplo tc^{99m} o I^{123}, o un marcador giratorio para resonancia magnética nuclear (RMN) (también conocida como diagnóstico por imágenes obtenidas mediante resonancia magnética), como yodo-123 nuevamente, yodo-131, indio-111, fluorina-19, carbono-13, nitrógeno-15, oxígeno-17, gadolinio, manganeso o hierro.

[0080] El radio u otros marcadores se pueden incorporar en el conjugado en formas conocidas. Por ejemplo, el péptido se puede biosintetizar o sintetizar mediante síntesis química de aminoácidos utilizando precursores adecuados de aminoácidos que contengan, por ejemplo, fluorina-19 en vez de hidrógeno. Los marcadores como tc^{99m} o I^{123}, Re^{186}, Re^{188} e In^{111} se pueden unir a través de un residuo de cisteína en el

péptido. El itrio-90 se puede unir a través de un residuo de lisina. El método IODOGEN (Fraker y col., (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 se puede utilizar para incorporar yodo-123. "*Monoclonal Antibodies in Immunoscintigraphy*" (Chatal, CRC Press 1989) describe otros métodos detalladamente.

[0081] Los conjugados del anticuerpo y el agente citotóxico se pueden realizar utilizando una variedad de agentes de unión a proteína bifuncionales como por ejemplo N-succinimidil-3-(2-piridilditio) propionato (SPDP), succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato (SMCC), iminotiolano (IT), derivados bifuncionales de imidoésteres (como el dimetiladipimidato HCl), ésteres activos (por ejemplo, disuccinimidil suberato), aldehídos (por ejemplo, glutaraldehídos), compuestos bis-azido (como bis (p-azidobenzoil) hexanodiamina), derivados de bis-diazonio (como bis-(p-diazoniobenzoil)-etilenodiamina), diisocianatos (por ejemplo, 2,6-diisocianato de tolueno) y compuestos bis-activos de fluoruro (como 1,5-difluoruro-2,4-dinitrobenceno). Por ejemplo, una inmunotoxina de ricina se puede preparar tal y como se describe en Vitetta y col., *Science*, 238:1098 (1987). El ácido triaminopentaacético 1-isotiocianatobencil-3-metildietileno marcado con carbono 14 (MX-DTPA) es un ejemplo de agente quelante para la conjugación de un radionucleótido a un anticuerpo. Véase el documento WO94/11026. El enlazador puede ser un "enlazador escindible" que facilita la liberación del fármaco citotóxico en la célula. Por ejemplo, se puede utilizar un enlazador lábil al ácido, un enlazador sensible a la peptidasa, un enlazador fotolábil, un enlazador de dimetilo o un enlazador que contenga disulfuri (Cari y col., *Cancer Research* 52:127-131, Patente de EE.UU. N. º 5.208.020).

[0082] Los compuestos de la invención contemplan expresamente, entre otros, el ADC preparado con reactivos reticulares: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC y sulfo-SMPB, y SVSB (sucinimidil-(4-vinilsulfona)benzoata) que se comercializan (por ejemplo, por Pierce Biotechnology, Inc., Rockford, IL., EE.UU). Véanse las páginas 467-498, 2003-2004 *Applications Handbook and Catalog.*

Preparación de los conjugados de anticuerpos con fármacos:

[0083] En los conjugados de anticuerpos con fármacos (ADC) de la invención, un anticuerpo (Ab) se conjuga con uno o más grúpos de fármacos (D), por ejemplo, grupos de fármacos de aproximadamente 1 a aproximadamente 20 por anticuerpo, a través de un enlazador (L). En una realización, el número de fracciones de fármaco (D) por anticuerpo es de aproximadamente 1 a aproximadamente 5, alternativamente, de aproximadamente 2 a aproximadamente 6, alternativamente, de aproximadamente 2 a aproximadamente 5, alternativamente, de aproximadamente 3 a aproximadamente 4 fracciones de fármaco por anticuerpo. Puesto que el número de fracciones de fármaco por anticuerpo es generalmente un promedio de todos los conjugados presentes en una población de un conjugado de fármaco y anticuerpo, el

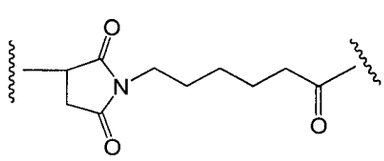
número de fracciones de fármaco por anticuerpo no puede ser un número entero. Los ADC de la formula I se pueden preparar por varios medios, empleando reacciones químicas orgánicas, condiciones y reactivos conocidos por los expertos, como: (1) una reacción de un grupo nucleofílico de un anticuerpo con un reactivo enlazador bivalente, para formar un Ab-L, mediante un enlace covalente, seguida de la reacción con una fracción de fármaco D; y (2) una reacción de un grupo nucleofílico de una fracción de fármaco con un reactivo enlazador bivalente, para formar D-L, mediante un enlace covalente, seguido de la reacción con el grupo nucleofílico del anticuerpo. En la presente memoria descriptiva se describen métodos adicionales para la preparación del ADC.

$$Ab–(L–D)_p \qquad \text{Fórmula I}$$

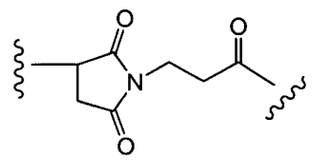
[0084] El enlazador puede estar formado por uno o más componentes enlazadores. Los componentes de enlace ejemplares incluyen: 6-maleimidocaproilo ("MC"), maleimidopropanoilo ("MP"), valina-citrulina ("val-cit"), alanina-fenilalanina ("ala-phe"), p-aminobensiloxicarbonil ("PAB"), N-Succinimidil 4-(2-piridiltio) pentanoato ("SPP"), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato ("SMCC'), y N-Succinimidil (4-yodo-acetil) aminobenzoato ("SIAB"). En una forma de realización, el ligando es valina-citrulina-p-aminobensiloxicarbonil ("vc-PAB") Los componentes enlazadores adicionales son conocidos en la materia y algunos se describen en la presente memoria descriptiva.

[0085] En ciertas formas de realización, el enlazador puede comprender residuos de aminoácidos. Los componentes enlazadores ejemplares de aminoácidos incluyen un dipéptido, un tripéptido, un tetrapéptido o un pentapéptido. Los dipéptidos ejemplares incluyen: valina-citrulina (vc o val-cit), alanina-fenilalanina (af o ala-phe). Los tripéptidos ejemplares incluyen: glicina-valina-citrulina (gl-val-cit) y glicina-glicina-glicina (gly-gly-gly). Los residuos de aminoácidos que comprenden un componente enlazador de aminoácidos incluyen aquellos que se producen de forma natural, como así también los aminoácidos menores y los análogos de aminoácidos que no se producen de forma natural, como la citrulina. Los componentes enlazadores de aminoácidos pueden designarse y optimizarse en su selectividad para la escisión enzimática mediante una enzima específica, por ejemplo, una proteasa asociada a un tumor, catepsina B, C y D o una proteasa plasmina.

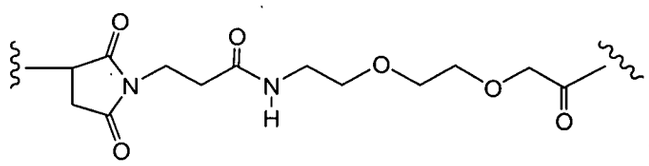
[0086] A continuación se muestran las estructuras ejemplares de los componentes enlazadores (donde la línea ondulada indica los sitios de enlace covalente a otros componentes del ADC)



MC

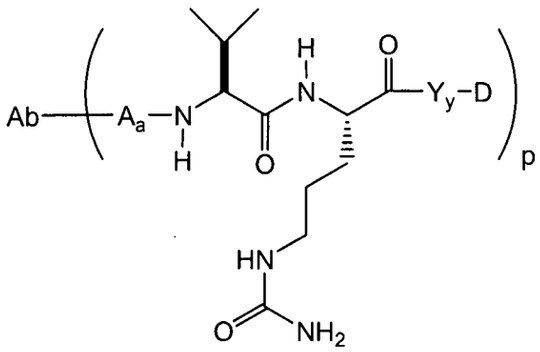


MP

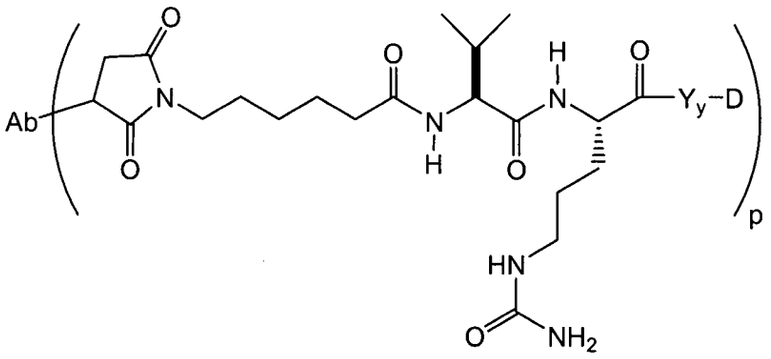


MPEG

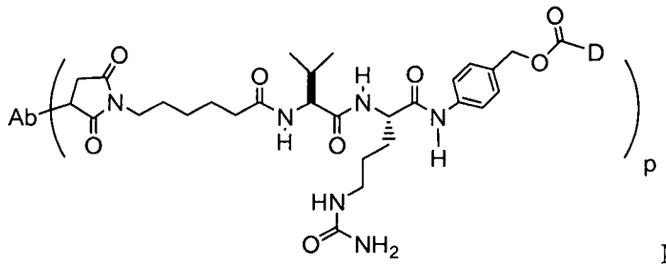
[0087] Los componentes enlazadores ejemplares adicionales y las abreviaciones incluyen (donde se muestra el anticuerpo (Ab) y el enlazador, y p es 1 a aproximadamente 8):



Val-cit



MC-val-cit



MC-val-cit-PAB

[0088] Los grupos nucleofílicos en los anticuerpos incluyen entre otros: (i) grupos amino N-terminal, (ii) grupos amino de la cadena lateral, por ejemplo lisina, (iii) grupos tiol de la cadena lateral, por ejemplo cisteína, y (iv) grupos amino o hidroxilo de azúcares donde el anticuerpo es glicosilado. Los grupos amino, tiol e hidroxilo son nucleofílicos y son capaces de reaccionar para formar uniones covalentes con grupos electrofílicos en las fracciones del enlazador y los reactivos enlazadores, que incluyen: (i) ésteres activos como los ésteres NHS, los ésteres HOBt, haloformos y haluro de ácido; (ii) haluros de alquilo y bencilo como las haloacetamidas; (iii) aldehídos, cetonas, carboxilo y grupos maleimidos. Ciertos anticuerpos contienen bisulfuros de intercadena reducible, es decir, puentes de cisteína. Los anticuerpos pueden convertirse en reactivos mediante conjugación con reactivos enlazadores mediante el tratamiento con un agente reductor como el DTT (ditiotreitol). Por lo tanto, cada puente de cisteína formará, en teoría, dos nucleofilos tiol reactivos. Los grupos nucleofilicos adicionales pueden introducirse en los anticuerpos a través de la reacción de las lisinas con 2-iminotiolano (reactivo de Traut), lo que da lugar a la conversión de una amina en un tiol. Los grupos tiol reactivos pueden introducirse en un anticuerpo (o un fragmento del mismo) mediante la introducción de uno, dos, tres, cuatro o más residuos de cisteína (por ejemplo preparando anticuerpos mutantes que comprendan uno o más residuos de aminoácidos de cisteínas no nativas).

[0089] Los conjugados de anticuerpos con fármacos de la invención también pueden producirse mediante la modificación del anticuerpo para introducir fracciones electrofílicas, que pueden reaccionar con sustituyentes nucleofílicos en el reactivo enlazador o el fármaco. Las azúcares de los anticuerpos glicosilados pueden oxidarse, por ejemplo, con reactivos oxidantes de periodato, a fin de formar grupos cetonas o aldehídos, los cuales pueden reaccionar con el grupo amina de los reactivos enlazadores o las fracciones del fármaco. Los grupos resultantes de amina base de Schiff pueden formar un enlace estable, o pueden reducirse, por ejemplo, mediante los reactivos de hidruro de boro para formar enlaces amina estables. En una forma de realización, la reacción de la porción carbohidratada de un anticuerpo glicosilado, ya sea con galactosa oxidasa o con metaperiodato de sodio, puede producir grupos de carbonilo (aldehídos y cetonas) en la proteína que pueden reaccionar con los grupos apropiados en el fármaco ("*Bioconjugate Techniques*" de Hermanson). En otra forma de realización, las proteínas que contienen residuos de serina o treonina N-terminal pueden reaccionar con metaperiodato de sodio, lo que da lugar a la producción de un aldehído en lugar del primer aminoácido (Geoghegan y Stroh, (1992) *Bioconjugate*

Chem. 3:138-146; patente de EE.UU. N.º 5362852). Se puede hacer que este aldehído reaccione con una fracción de fármaco o un nucleófilo enlazador.

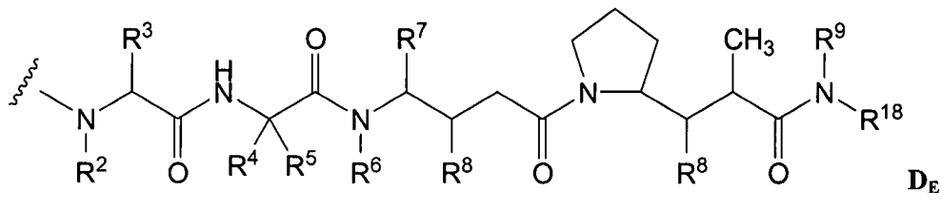
[0090] Del mismo modo, los grupos nucleofílicos en una fracción del fármaco incluyen los siguientes compuestos: los grupos amino, tiol, hidroxilo, hidracida, oxima, hidracina, tiosemicarbazona, carboxilato de hidracina y aril-hidracida, capaces de reaccionar para formar enlaces covalentes con grupos electrofílicos en las fracciones enlazadoras y reactivos enlazadores, que incluyen: (i) ésteres activos como los ésteres NHS, los ésteres HOBt, haloformos y haluro de ácido; (ii) haluros de alquilo y bencilo como las haloacetamidas; (iii) aldehídos, cetonas, carboxilo y grupos maleimidos.

[0091] Se encuentran métodos de conjugación de fracciones enlazador-fármaco a proteínas que actúan sobre las células, tales como los anticuerpos, la inmonoglobulina o los fragmentos de la misma en, por ejemplo, patente de EE.UU. N.º 5.208.020; patente de EE.UU. N.º 6.441.163; documentos WO2005037992, WO2005081711 y WO2006/034488, los cuales se incorporan expresamente en su totalidad a modo de referencia.

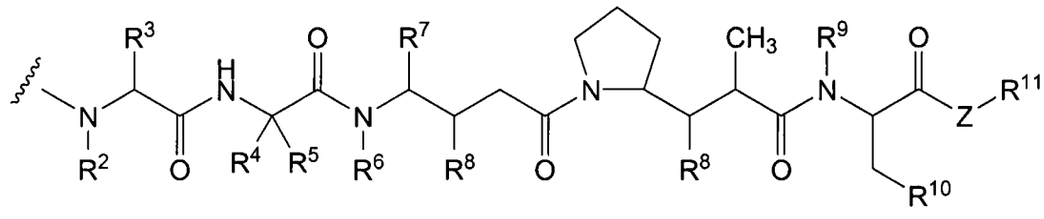
[0092] Alternativamente, es posible realizar una fusión de proteínas que contengan el anticuerpo y el agente citotóxico, por ejemplo mediante técnicas recombinantes o síntesis de péptidos. La longitud del ADN puede incluir regiones respectivas que codifiquen las dos porciones del conjugado, ya sea adyacentes entre sí o separados por una región que codifica un péptido de enlace, el cual no destruye las propiedades deseadas del conjugado.

[0093] En otra forma de realización, el anticuerpo puede conjugarse con un "receptor" (como la estreptavidina) y actuar previamente contra el tumor, donde se administra al paciente el conjugado de anticuerpo con receptor, luego se elimina de la circulación el conjugado no unido utilizando un agente de eliminación, y posteriormente se administra un "ligando" (por ejemplo, avidina) que se conjuga con un agente citotóxico (por ejemplo, un radionucleótico).

[0094] En una forma de realización del inmunoconjugado, el agente citotóxico, D, es una auristatina de fórmula D_E o D_F



D_E



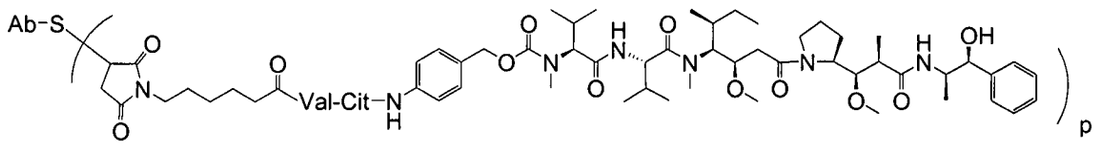
D_F

y donde R^2 y R^6 son, cada uno, metilo; R^3 y R^4 son cada uno isopropilo; R^7 es sec butilo, cada R^8 se selecciona independientemente entre CH_3, O-CH_3, OH, y H; R^9 es H; R^{10} es arilo; Z es –O– o –NH–; R^{11} es H, C_1-C_8 alquilo o $-(CH_2)_2-O-(CH_2)_2-O-(CH_2)_2-O-CH_3$; y R^{18} es $-C(R^8)_2-C(R^8)_2-$arilo; y

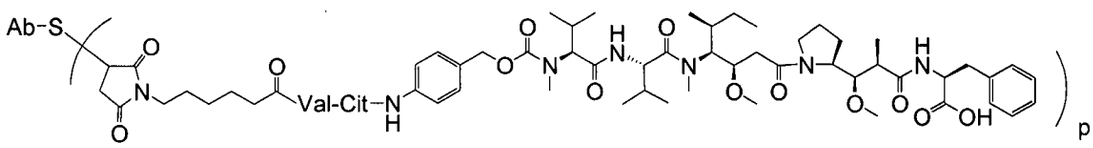
(d) p oscila entre aproximadamente 1 y 8.

[0095] Se proporcionan también las siguientes formas de realización para cualquiera de los inmunoconjugados anteriores. En una forma de realización, un inmunoconjugado tiene actividad destructora de células *in vitro* o *in vivo*. En una forma de realización, el enlazador se une al anticuerpo a través de un grupo tiol en el anticuerpo. En una forma de realización, el enlazador es escindible mediante una proteasa. En una forma de realización, el enlazador comprende un dipéptido val-cit. En una forma de realización, el enlazador comprende una unidad p-aminobenzilo. En una forma de realización, la unidad p-aminobenzilo está situada entre el fármaco y un sitio de escisión de la proteasa en el enlazador. En una forma de realización, la unidad p-aminobenzilo es un p-aminobenziloxicarbonilo (PAB). En una forma de realización, el enlazador comprende 6-maleimidocaproilo. En una forma de realización, 6-maleimidocaproilo está situado entre el anticuerpo y un sitio de escisión de la proteasa en el enlazador. Las formas de realización anteriores pueden darse por separado o combinadas entre sí.

[0096] En una forma de realización, el fármaco se selecciona entre MMAE y MMAF. En una forma de realización, el inmunoconjugado tiene la fórmula



donde Ab es cualquiera de los anticuerpos anti-STEAP-1 anteriores, S es un átomo de sulfuro y p oscila entre alrededor de 2 y alrededor de 5. En una forma de realización, el inmunoconjugado tiene la fórmula



donde Ab es cualquiera de los anticuerpos anti-STEAP-1 anteriores, S es un átomo de sulfuro y p oscila entre aproximadamente 1 y aproximadamente 6 , entre aproximadamente 2 y aproximadamente 5, entre aproximadamente 2 y aproximadamente 6, entre aproximadamente 2 y aproximadamente 4, entre aproximadamente 2 y aproximadamente 3, entre aproximadamente 3 y aproximadamente 4, entre aproximadamente 3 y aproximadamente 5, entre aproximadamente 3 y aproximadamente 6, entre aproximadamente 4 y aproximadamente 6.

Métodos de diagnóstico por imágenes del anticuerpo marcado:

[0097] En otra forma de realización de la invención, los anticuerpos diseñados con cisteína pueden marcarse mediante tiol de cisteína con radionucleidos, tintes fluorescentes, grupos de sustratos activadores de la bioluminiscencia, grupos de sustratos activadores de la quimioluminiscencia, enzimas y otros marcadores de detección para experimentos con imágenes con aplicaciones de diagnóstico, farmacodinámica y terapéuticas. Generalmente, el anticuerpo diseñado con cisteína marcado, es decir, "biomarcador" o "sonda", se administra por inyección, perfusión o ingesta oral a un organismo vivo, por ejemplo, un ser humano, un roedor u otro animal pequeño, un órgano perfundido o una muestra de tejido. La distribución de la sonda se detecta durante un período de tiempo y se representa mediante una imagen.

Artículos de fabricación:

[0098] En otra forma de realización de la invención, se proporcionan artículos fabricados o "kit" que contienen materiales útiles para el tratamiento de los trastornos descritos anteriormente. El elemento de fabricación comprende un envase y una etiqueta o prospecto en el envase o asociado a él. Entre los envases adecuados se encuentran, por ejemplo, botes, viales, jeringas, ampollas, etc. Los envases pueden estar hechos de diversos materiales, como vidrio o plástico. El envase contiene una composición de conjugado de anticuerpo con fármaco (ADC) que es eficaz en el tratamiento de la enfermedad y puede tener un puerto de acceso estéril (por ejemplo, el envase puede ser una bolsa para solución intravenosa o un vial con un tapón perforable por una aguja hipodérmica). Al menos un agente activo de la composición es un conjugado de anticuerpo con fármaco. La etiqueta o prospecto indica que la composición se utiliza para el tratamiento de la afección concreta, tal como el cáncer. De modo alternativo o adicional, el elemento de fabricación puede comprender un segundo (o tercer) envase que contiene una solución farmacéuticamente aceptable, como agua bacteriostática para inyección (BWFI), una solución salina fosfatada, una solución de Ringer y una solución de dextrosa. También puede incluir otros materiales deseables desde el punto de vista comercial y del usuario, incluidas otras soluciones, diluyentes, filtros, agujas y jeringas.

Composiciones farmacéuticas:

[0099] En un aspecto, se proporciona una composición farmacéutica que comprende cualquiera de los inmunoconjugados anteriores y un vehículo farmacéuticamente aceptable. En un aspecto, se

proporciona un método para el tratamiento de un trastorno proliferativo de la células de próstata, pulmón, colon, vejiga o de ovario, o sarcoma de Ewing, en el cual dicho método comprende la administración a un sujeto de la composición farmacéutica. En una forma de realización, el cáncer de próstata, pulmón, colon, vejiga y ovario y un trastorno de proliferación celular de sarcoma de Ewing es una metastasis de un cancer primario de próstata, pulmón, colon, vejiga u ovario o sarcoma de Ewing. En una forma de realización, el trastorno de proliferación celular se asocia a un aumento de la expresión de STEAP-1 en la superficie de una célula.

[0100] En un aspecto, se proporciona un método para inhibir la proliferación celular, en el cual dicho método comprende la exposición de una célula a cualquiera de los inmunoconjugados anteriores en condiciones que favorezcan la unión del inmunoconjugado a STEAP-1. En una forma de realización, la célula de próstata, pulmón, colon, vejiga o de ovario, o sarcoma de Ewing, es una célula tumoral. En una realización, la célula tumoral es una célula de un tumor de próstata, pulmón, colon, vejiga u ovario o de un sarcoma de Ewing de un mamífero que sufre o se sospecha que sufre un trastorno de proliferación celular de próstata, pulmón, colon o vejiga o de un sarcoma de Ewing que incluye, aunque sin limitarse a ella, una metástasis de un tumor cancerígeno primario de próstata, pulmón, colon o vejiga o de un sarcoma de Ewing. En una realización, la célula de próstata, pulmón, colon o vejiga o del sarcoma de Ewing es un xenotrasplante. En una forma de realización, la exposición se produce *in vitro*. En una forma de realización, la exposición se produce *in vivo*.

[0101] En un aspecto, se proporciona un método de utilización del anticuerpo anti-STEAP-1 para efectuar un ensayo de suero soluble STEAP-1 en un mamífero que experimenta trastorno de la proliferación celular de próstata, pulmón o colon (o metástasis de la incidencia primaria de dicho trastorno), midiendo la progresión clínica o la regresión de las enfermedades, o evaluando la carga o recurrencia del tumor.

BREVE DESCRIPCIÓN DE LAS FIGURAS

[0102] La patente o archivo de solicitud contiene al menos, un dibujo ejecutado a color. Las copias de esta patente o publicación de solicitud de patente con dibujo(s) a color, se proporcionará por la Oficina después de la petición y pago de las tasas necesarias.

[0103] **Figura 1** describe la secuencia de aminoácidos de la STEAP-1 humano (identificador de secuencia n.º: 1) alineados con STEAP-1 del ratón y del mono cinomolgus (identificador de secuencias n.º 2 y 3 respectivamente). Los dominios extracelulares 1, 2 y 3 están identificados y marcados por recuadros sombreados.

[0104] Figuras 2A y 2B: La **Figura 2A** describe la secuencia de aminoácidos de la región variable de la cadena ligera del anticuerpo anti-STEAP-1 120.545 murino que se alinea con el anticuerpo quimérico (quimera 120) y anticuerpo humanizado (injerto 120) y con la secuencia del subgrupo humano III. Las CDR se muestran en un recuadro (CDR-L1, CDR-L2, and CDR-L3). Las secuencias que agrupan las CDR corresponden a las secuencias de estructuras (de FR-L1 a FR-L4). Las secuencias se enumeran de acuerdo con la numeración de Kabat. Las CDR de Kabat, Chothia y de contacto se indican en las CDR recuadradas. La **Figura 2B** describe la secuencia de aminoácidos de la región variable de la cadena pesada del anticuerpo anti-STEAP-1 murino (120.545) que se alinea con el anticuerpo quimérico (quimera 120) y el anticuerpo humanizado (injerto 120) y con la secuencia kappa humana I. Se prepararon variantes humanizadas 24, 37, 48, 67 y 37/48, 67, 71 y 78 realizando los siguientes cambios de aminoácidos: A24V, V37I, V48M, F67I y L78F en la cadena pesada del anticuerpo del injerto 120. Las CDR se muestran en recuadros. Las secuencias FR-H1, FR-H2, FR-H3 y FR-H4 agrupan las CDR (CDR-H1, CDR-H2, and CDR-H3). Las secuencias se enumeran de acuerdo con la numeración de Kabat. Las CDR de Kabat, Chothia y de contacto se indican en las CDR recuadradas.

[0105] Las **Figuras 3A y 3B** muestran secuencias de estructuras de consenso humanas pesadas variables (VH) del aceptor ejemplares para utilizar en la práctica de la invención con identificadores de secuencia tal como se indica a continuación, donde los números de identificadores de secuencia de estructuras se enumeran en el orden de FR-H1, FR-H2, FR-H3, FR-H4:

- estructura "A" de consenso del subgrupo I humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 26, 27, 28, 29).

- estructuras "B", "C" y "D" de consenso del subgrupo I humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 30, 31, 28, 29; identificadores de secuencia n.º: 30, 31, 32, 29; e identificadores de secuencia n.º: 30, 31, 33, 29).

- estructura "A" de consenso del subgrupo II humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 34, 35, 36, 29).

- estructuras "B", "C" y "D" de consenso del subgrupo II humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 37, 38, 36, 29; identificadores de secuencia n.º: 37, 38, 39, 29; e identificadores de secuencia n.º: 37, 38, 40, 29).

- estructura "A" de consenso del subgrupo III humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 41, 42, 43, 29).

- estructuras "B", "C" y "D" de consenso del subgrupo III humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 44, 45, 43, 29; identificadores de secuencia n.º: 44, 45, 46, 29; e identificadores de secuencia n.º: 44, 45, 46, 29).

- estructura "A" del aceptor 1 humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 48, 42, 49, 29).

- estructuras "B" y "C" del aceptor humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 44, 45, 49, 29; e identificadores de secuencia n.º: 44, 45, 50, 29).

- estructura "A" del aceptor 2 humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 48, 42, 51, 29).

- estructuras "B", "C" y "D" del aceptor 2 humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 44, 45, 51, 29; identificadores de secuencia n.º: 44, 45, 52, 29; e identificadores de secuencia n.º: 44, 45, 53, 29).

[0106] Las **Figuras 4A y 4B** muestran secuencias de estructuras de consenso humanas ligeras variables (VL) del aceptor ejemplares para utilizar en la práctica de la invención con identificadores de secuencia tal como se indica a continuación:

- estructura de consenso del subgrupo I-1 kappa humano VL (κv1-1): identificadores de secuencia n.º: 54, 55, 56, 57

- estructura de consenso del subgrupo I kappa humano de VL (κv1): identificadores de secuencia n.º: 54, 58, 56, 57

- estructura de consenso del subgrupo II kappa humano de VL (κv2): identificadores de secuencia n.º: 58, 59, 60, 57

- estructura de consenso del subgrupo III kappa humano de VL (κv3): identificadores de secuencia n.º: 61, 62, 63, 57

- estructura de consenso del subgrupo IV kappa humano de VL (κv4): identificadores de secuencia n.º: 64, 65, 66, 57.

[0107] La **Figura 5** representa las alineaciones de las secuencias de la región Fc de IgG humana de secuencia nativa, humIgG1 (alotipos no A, identificador de secuencia n.º: 85; y alotipos A, donde la secuencia de aminoácidos SREEM en el identificador de secuencia n.º: 85 cambia a SRDEL), humIgG2 (identificador de secuencia n.º 86), humIgG3 (identificador de secuencia n.º: 87) y humIgG4 (identificador de secuencia n.º: 88) con diferencias entre las secuencias marcadas con asteriscos. Los números sobre las secuencias representan el sistema de numeración EU. También se muestra un ejemplo de la región constante kappa.

[0108] Las **Figuras 6A-6D** representan un análisis FACS (Separador Celular Activado por Fluoresceína) normalizado para el nivel de expresión de cada anticuerpo o variante en el fago. La Figura 6A muestra variantes de FACS en el STEAP-1 con expresión celular (LB50) para cuatro ejemplos de

anticuerpos. La Figura 6B muestra variantes de FACS en el STEAP-1 sin expresión celular (S408) para varios anticuerpos como se indica en la figura y en el Ejemplo 1. Las Figuras 6C y 6D son alineaciones de variantes de FACS después de la normalización para los niveles de expresión en fagos.

[0109] Las **Figuras 7A-7F** representan gráficamente análisis FACS que muestran la unión de anticuerpos 24 anti-STEAP 1 de versión murina, quimérica y humanizada a STEAP-1 humano expresado en la superficie celular. Las Figuras 7A-7C indican que los anticuerpos murina 120, quimérica 120 y humanizada 120 v.24 del anti-STEAP-1 se unen al STEAP-1 humano y de mono cynomolgus, pero no al STEAP-1 de ratón. Las Figuras 7D-7F son diagramas de FACS que muestran la unión de anticuerpos (clon 67) de murina 120, quimérica 120 y humanizada 120 v. 24 a SEAP-1 humano expresado en la superficie celular. El STEAP-1 exógeno se expresó de manera estable en células 293 (denominadas células LB50) y en células PC3 (denominadas células PS5.4) (Figuras 7D y 7E), y se expresó endógenamente en células LNCaP BR (Figura 7F).

[0110] **Figuras 8A y 8B.** La **Figura 8A** es un gráfico que muestra que la administración de 120-MC-vc-PAB-MMAE anti-STEAP murino en 3 mg/kg fue eficaz en un modelo xenotrasplantado (células LNCaP-Ner) de tumor de próstata. Véase el Ejemplo 4. La **Figura 8B** es un gráfico que muestra que la administración de una dosis única del anticuerpo 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg) y 120v.24-MC-MMAF (12 mg/kg) anti-STEAP-1 humanizado, y de 120 MC-vc-PAB-MMAE (3 mg/kg) anti-STEAP-1 quimérico resultó eficaz en un modelo de tumor de próstata trasplantado de célula LNCaP. Véase el Ejemplo 4.

[0111] La **Figura 9** es un gráfico que muestra que la administración del anticuerpo 120 MC-vc-PAB-MMAE quimérico anti-STEAP-1 (abreviado anti-STEAP vcMMAE) en 3 mg/kg, o de un MC-MMAF 120 quimérico STEAP-1 (abreviado anti-STEAP mcMMAF) en 6 mg/kg resultó eficaz en un modelo xenotrasplantado de cáncer de próstata de ratón beige SCID castrado trasplantado con células LNCaP. Véase el Ejemplo 4.

[0112] La **Figura 10** es un gráfico que muestra que la administración del anticuerpo 120 MC-vc-PAB-MMAE quimérico anti-STEAP-1 (abreviado anti-STEAP vcMMAE (en 3 mg/kg) resultó eficaz en un modelo xenotrasplantado de cáncer de próstata de ratón macho beige SCID (andrógeno dependiente) trasplantado con células LuCap 77. Véase el Ejemplo 4.

[0113] La **Figura 11** es un gráfico que muestra que la administración del anticuerpo 120v.24-MC-vc-PAB-MMAE humanizado anti-STEAP-1 en 3 mg/kg, y del anticuerpo 120v.24-MC-MMAF humanizado anti-STEAP-1 en 6 mg/kg y 12 mg/kg a ratón beige SCID castrado trasplantado con tumor de próstata LuCap35V resultó eficaz en relación con los controles. Véase el Ejemplo 4.

[0114] La **Figura 12** es un diagrama que representa al STEAP-1 embebido en una membrana celular. La unión del anticuerpo 120 anti-STEAP-1 depende de la conformación y no reconoce a un epítopo lineal de STEAP-1 (péptido revelado en SEC ID NO. 102).

[0115] Las **Figuras 13A-13D** muestran al STEAP-1 expresado sobre la superficie de las células cuando es detectado mediante inmunohistoquímica. La **Figura 13A** muestra un teñido inmunohistoquímico de células 293 que expresan el STEAP-1 exógeno sobre la superficie celular. La **Figura 13B** muestra un teñido inmunohistoquímico de células PC3 que expresan el STEAP-1 exógeno sobre la superficie celular. La **Figura 13C** muestra un teñido inmunohistoquímico de células LNCaP que expresan el STEAP-1 endógeno sobre la superficie celular. La **Figura 13D** muestra un teñido inmunohistoquímico de células LuCAP 77 que expresan el STEAP-1 endógeno sobre la superficie celular.

[0116] Las **Figuras 14A-14E** son gráficos que muestran la eficacia relativa del anticuerpo 120v.24-MCMMAF anti-STEAP-1 y del anticuerpo 120v.24-MC-vc-PAB-MMAE anti-STEAP-1 para destruir al STEAP-1 que se expresa en células in vitro. Las células PS5.4 (**Figura 14A**) son células PC3 transformadas con un vector que codifica a STEAP-1 tal como ese STEAP-1 se expresa en la superficie celular. Las células LB50 (**Figura 14B**) son células 293 transformadas con un vector que codifica a STEAP-1 tal como ese STEAP-1 se expresa en la superficie celular. Las células LNCaP (**Figura 14C**) expresan endógenamente a STEAP-1. "PC3 vec" (**Figura 14D**) y "293 vec" (**Figura 14E**) se refieren a las células 293 y PC3, respectivamente, transformadas con un vector de control.

[0117] La **Figura 15** muestra las representaciones de los conjugados de anticuerpo anti-STEAP-1 diseñado con cisteína (ADC) con fármacos, donde una fracción del fármaco se une a un grupo diseñado con cisteína en: La cadena ligera (LC-ADC); la cadena pesada (HC-ADC) y la región Fc (Fc-ADC).

[0118] La **Figura 16** muestra los pasos para: (i) reducir los aductos de disulfuros de cisteína, las intercadenas y los disulfuros de las intercadenas en un anticuerpo anti-STEAP-1 diseñado con cisteína (ThioMab) con un agente TCEP reductor (tris(2-carboxietil)fosfino hidrocloruro); (ii) oxidar parcialmente, es decir, reoxidar para reformar la intercadena y los disulfuros de intercadenas con dhAA (ácido dehidroascórbico); y (iii) lograr la conjugación del anticuerpo reoxidado con un enlazador a fármaco intermedio para formar un conjugado de anticuerpo anti-STEAP-1 diseñado con cisteína con fármacos (ADC).

[0119] Las **Figuras 17AC** muestran los sitios de sustitución de aminoácidos realizados para generar anticuerpos anti-STEAP-1 diseñados con cisteína (tio-anticuerpos monoclonales). La **Figura 17A** muestra la variante tio-LC de V205C con la correspondiente numeración secuencial y la numeración estandarizada de acuerdo con el sistema Kabat (SEC ID NO. 103 – 113 respectivamente en orden de aparición). La **Figura 17B** muestra la variante tio-HC de A118C con la correspondiente numeración secuencial y la numeración estandarizada de acuerdo con el sistema de la UE (SEC ID NO. 114 – 124 respectivamente en orden de aparición). La **Figura 17C** muestra la variante tio-Fc de S400C con la correspondiente numeración secuencial y la numeración estandarizada de acuerdo con el sistema de la UE (SEC ID NO. 125 – 135 respectivamente en orden de aparición).

Las **Figuras 18A-F** representan análisis FACS que muestran que los conjugados de fármaco y anticuerpo tio anti-STEAP-1 (TDC) conservan la capacidad de unión a STEAP-1 expresado en la superficie celular. Las **Figuras 18A-18C** son diagramas FACS que muestran la unión de 120-vc-PAB-MMAE (LCV205C) humano tio anti-STEAP-1 (abreviado como huSteap1 TDC (L205C) vcE y120-vc-PAB-MMAE (HCA118C) (abreviado como huSteap1 TDC (HCA118C) vcE) humano tio a STEAP-1 expresado en la superficie celular. El STEAP-1 exógeno se expresó de manera estable en células 293 (denominadas células LB50) y en células PC3 (denominadas células PS5.4) (**Figuras 18A y 18B**), y se expresó endógenamente en células LNCaP BR (**Figura 18C**). Las **Figuras 18D, 18E y 18F** son alineaciones de las variantes FACS que se muestran en las **Figuras 7A, 7B y 7C**, respectivamente.

[0120] Las **Figuras 19A-C** muestran la eficacia relativa de los conjugados de fármaco y anticuerpo (TDC) 120-vc-PAB-MMAE (LCV205C) humano tio anti-STEAP-1 (LCV205C) (abreviado como huSteap1 TDC (L205C) vcE) y120-vc-PAB-MMAE (HCA118C) (abreviado como huSteap1 TDC (HCA118C) vcE) humano tio para destruir la expresión de STEAP-1 en células in vitro. Las células LB50 (**Figura 19A**) son células 293 transformadas con un vector que codifica a STEAP-1 tal como ese STEAP-1 se expresa en la superficie celular. Las células PS5.4 (**Figura 19B**) son células PC3 transformadas con un vector que codifica a STEAP-1 tal como ese STEAP-1 se expresa en la superficie celular. Las células LNCaP (**Figura 19C**) expresan endógenamente a STEAP-1.

[0121] La **Figura 20** es un gráfico que muestra que la administración del anticuerpo 120-vc-PAB-MMAE humano tio anti-STEAP-1 (HCA118C) (abreviado hu Steap1 HC TDC vcE) en 3 mg/kg resultó eficaz en relación con los controles en un modelo xenotrasplantado de cáncer de próstata de ratón macho beige SCID (andrógeno dependiente) trasplantado con células LNCaP. Véase el Ejemplo 8.

[0122] La **Figura 21** es un gráfico que muestra que la administración de TDC anti-STEAP-1 120-vc-PAB-MMAE humano tio (HCA118C) (abreviado como hu Steap1 HC TDC vcE) en 3 mg/kg o de 120-MC-MMAF humano tio (HCA118C) (abreviado como hu Steap1 HC TDC mcF) en 1, 3 ó 6 mg/kg resultó eficaz en relación con los controles en un modelo xenotrasplantado de cáncer de próstata de ratón beige SCID macho (andrógeno dependiente) trasplantado con células LNCaP. Véase el Ejemplo 8.

[0123] La **Figura 22** es un gráfico que muestra que la administración de 120-vc-PAB-MMAE humano tio anti-STEAP-1 TDC (HCA118C) (abreviado hu Steap1 HC TDC vcE) en 3 mg/kg o de 120-MC-MMAF (HCA125C) (abreviado como hu Steap1 HC TDC mcF) humano tio en 3 ó 6 mg/kg resultó eficaz en relación con los controles en un modelo xenotrasplantado de cáncer de próstata de ratón beige SCID castrado trasplantado con tumor de próstata LuCaP 35V. Véase el Ejemplo 8.

[0124] La **Figura 23** muestra los sitios de sustitución de aminoácido hechos para generar el anticuerpo anti-STEAP-1 diseñado por ingeniería de cisteína (tio-mAb), designado "Simmons IV", o simplemente "SGIV". La secuencia de aminoácido de la cadena ligera de SGIV (SEC ID NO:90), se

muestra en alineamiento con la cadena ligera del anticuerpo mu 120 (SEC ID NO. 5) y anticuerpo 120.v24 (SEC ID NO:91). La variante tio-LC de SGIV con la numeración secuencial correspondiente y numeración estandarizada de conformidad con el sistema Kabat, se muestra alineada con el anticuerpo precursor mu 120, así como también con la variante tio-LC de 120.v24 con numeración secuencial correspondiente y numeración estandarizada, de conformidad con el sistema Kabat. Las CDR están enmarcadas (CDR-L1, CDR-L1 y CDR-L3). Las secuencias que encierran las CDR son las secuencias de estructura (FR-L1 a FR-L4). Las secuencias son numeradas de conformidad con la numeración de Kabat. Las CDR de Kabat, Chothia y de contacto, están indicadas alrededor de las CDR enmarcadas. Véase Ejemplo 9.

[0125] La **Figura 24** muestra los sitios de sustituciones de aminoácido de estructura para generar varias variantes de anticuerpos anti-STEAP-1 diseñados por ingeniería de cisteína (tio-mAb), de los anticuerpos SGIV y 120v.24. La secuencia de aminoácido de la cadena ligera de SGIV (SEC ID NO. 136) se muestra con numeración estandarizada de conformidad con el sistema de Kabat, en alineamiento con variantes LS.VLVH1 (SEC ID NO:92); LS.VLVH2 (SEC ID NO:93); LS.Q (SEC ID NO:94); y LS.CH1 (SEC ID NO:95). La secuencia de aminoácido de la cadena ligera de 120.v24 (SEC ID NO. 137) con numeración estandarizada de conformidad con el sistema Kabat, se muestra en alineamiento con variantes ED.FW1 (SEC ID NO:96); ED.FW2 (SEC ID NO:97); ED.FW3 (SEC ID NO:98); ED.all (SEC ID NO:99); ED.Pro (SEC ID NO:100); y ED.pl (SEC ID NO:101). Las CDR están enmarcadas. Las secuencias son numeradas de conformidad con la numeración de Kabat. Véase Ejemplo 9.

[0126] La **Figura 25** muestra trazos Scatchard de enlace anticuerpo a STEAP-1 expresados en la superficie de las células LNCaP.BR. Las muestras duplicadas fueron medidas usando el anticuerpo 120.v24 (Figuras 25(A)-(D)) y la variante SGIV (Figuras 25(E)-(H)). Véase Ejemplo 9.

[0127] La **Figura 26** muestra trazos Scatchard de enlace anticuerpo a STEAP-1 expresados en la superficie de las células 293.LB50. Las muestras duplicadas fueron medidas usando el anticuerpo 120.v24 (Figuras 26(A)-(D)) y la variante SGIV (Figuras 26(E)-(H)). Véase Ejemplo 9.

[0128] La **Figura 27** es una tabla que compara las afinidades de enlace promedio, medidas por el análisis Scatchard, para mu 1789, mu 120, quimera Fc, anticuerpos humanizados 120.v24, tio-120.v24 y tio-SGIV en células PC-3-PS5.4, 293-LB50 y LNCaP-BR, así como también en células 293 que expresan temporalmente STEAP-1. Véase Ejemplo 9.

[0129] La Figura 28 representa un análisis FACS que muestra cambios FACS en células establemente transfectadas con STEAP-1 (293 STEAP-1 LB48, 293 STEAP-1 LB50 y 293 STEAP-1 LB53), con muestras de anticuerpo SGIV y 120.v24. Véase Ejemplo 9.

[0130] La Figura 29 muestra el titulador de anticuerpo observado en diferentes colectadas de células que producen anticuerpo SGIV o 120.v24.

DESCRIPCIÓN DETALLADA DE LAS FORMAS DE REALIZACIÓN DE LA INVENCIÓN

[0131] Se proporcionan anticuerpos aislados que se unen a STEAP-1. Se proporcionan, además, inmunoconjugados que comprenden anticuerpos anti-STEAP-1. Los anticuerpos e inmunoconjugados de la invención son útiles, como por ejemplo, para el diagnóstico o tratamiento de trastornos asociados a una expresión alterada, como por ejemplo, una expresión aumentada, de STEAP-1. En determinadas formas de realización, los anticuerpos o inmunoconjugados de la invención son útiles para el diagnóstico o tratamiento de un trastorno de proliferación celular, como un tumor o cáncer. En ciertas realizaciones, STEAP-1 se expresa en el tumor o cáncer del tejido de la próstata, del pulmón o del colon. En determinadas formas de realización, los anticuerpos o inmunoconjugados de la invención son útiles para la detección de STEAP-1, por ejemplo, STEAP-1 expresado en la superficie celular. En determinadas formas de realización, los anticuerpos o inmunoconjugados de la invención son útiles para la detección de la expresión STEAP-1 en la superficie de las células normales y/o tumorales o cancerosas de tejido de próstata, pulmón o colon.

[0132] Se proporcionan polinucleótidos que codifican anticuerpos anti-STEAP-1. Se proporcionan vectores que comprenden polinucleótidos que codifican anticuerpos anti-STEAP-1, así como células hospederas que comprenden dichos vectores. También se proporcionan composiciones, incluyendo las formulaciones farmacéuticas, que comprenden uno o más de los polinucleótidos, anticuerpos anti-STEAP-1 o inmunoconjugados de la invención.

[0133] Se ofrecen métodos para el tratamiento de un trastorno de proliferación celular incluido, pero sin limitarse al mismo, un tumor o un cáncer, con un anticuerpo anti-STEAP-1, un conjugado de fármaco y anticuerpo o un inmunoconjugado. Dichos métodos incluyen, aunque sin limitarse al mismo, el tratamiento de tumor o cáncer de próstata, pulmón o colon en un mamífero. Se ofrecen métodos para la detección de la expresión de STEAP-1 en un tejido celular utilizando un anticuerpo anti-STEAP-1, un conjugado de fármaco y anticuerpo o un inmunoconjugado. Dichos métodos incluyen, aunque sin limitarse a la misma, la detección de la expresión de STEAP-1 en, como un ejemplo no limitante, una célula normal, una célula tumoral o una célula cancerosa de próstata, pulmón o colon.

Técnicas generales

[0134] Las técnicas y procedimientos descritos o remitidos en la presente memoria descriptiva por lo general se conocen bien y se emplean habitualmente con la metodología tradicional utilizada por los expertos en la materia, como por ejemplo, las metodologías ampliamente utilizadas descritas en Sambrook y col., *Molecular Cloning: A Laboratory Manual* 3.ª edición (2001) Cold Spring Harbor Laboratory Press,

Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F. M. Ausubel, y col. eds., (2003)); la serie *Methods in Enzymology* (Academic Press, Inc.): *Pcr 2: A Practical Approach* (M. J. MacPherson, B. D. Hames y G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual, and Animal Cell Culture* (R. I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M. J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R. I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather y P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J. B. Griffiths, y D. G. Newell, eds., 1993-8) J. Wiley y Sons; *Handbook of Experimental Immunology* (D. M. Weir y C. C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller y M. P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis y col., eds., 1994); *Current Protocols in Immunology* (J. E. Coligan y col., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C. A. Janeway y P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd y C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow y D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti y J. D. Capra, eds., Harwood Academic Publishers, 1995); y *Cancer: Principles and Practice of Oncology* (V. T. DeVita y col., eds., J.B. Lippincott Company, 1993).

DEFINICIONES Y ABREVIATURAS

Definiciones

[0135] Un anticuerpo "aislado" es aquél que se ha identificado y separado, y/o recuperado, a partir de un componente de su entorno natural. Los componentes contaminantes de su entorno natural son materiales que podrían interferir en la investigación, el diagnóstico o los usos terapéuticos del anticuerpo y pueden incluir enzimas, hormonas y otros solutos proteináceos y no proteináceos. En algunas formas de realización, un anticuerpo se purificará (1) en más del 95% de su peso tal como se determina, por ejemplo, por el método de Lowry, y en algunas formas de realización, en más del 99% de su peso, (2) en un grado suficiente para obtener al menos 15 residuos de una secuencia de aminoácidos N-terminal o interna mediante, por ejemplo, un secuenciador de taza giratoria, o (3) hasta alcanzar la homogeneidad mediante SDS-PAGE, en condiciones reductoras o no reductoras, empleando, por ejemplo, la tinción con azul de Coomassie o plata. Los anticuerpos aislados incluyen el anticuerpo *in situ* dentro de células recombinantes, ya que al menos un componente del entorno natural del anticuerpo no estará presente. En cualquier caso, normalmente, el anticuerpo aislado se preparará mediante al menos una etapa de purificación.

[0136] Una molécula de ácido nucleico "aislada" es una molécula de ácido nucleico que se separa de al menos una de las otras moléculas de ácido nucleico con las que se asocia habitualmente, por ejemplo, en su entorno natural. Una molécula aislada de ácido nucléico incluye, además, una molécula de ácido

nucleico contenida en células que habitualmente expresan la molécula de ácido nucleico, pero la molécula de ácido nucleico está presente extracromosómicamente o en una ubicación cromosómica diferente de su ubicación cromosómica natural.

[0137] Por "purificada" se entiende una molécula que está presente en una muestra a una concentración de al menos el 95% de su peso, o al menos el 98% del peso de la muestra en la que está contenida.

[0138] Las frases "sustancialmente similar" o "sustancialmente el/la mismo(a)", tal y como se usan en la presente memoria descriptiva, indican un grado suficientemente elevado de similitud entre dos valores numéricos (por ejemplo, uno asociado a un anticuerpo de la invención y el otro asociado a un anticuerpo de control/referencia), de forma que alguien experto en la materia consideraría la diferencia entre los dos valores de escasa o ninguna significación estadística o biológica, o ambos, dentro del contexto de la característica biológica medida por dichos valores (por ejemplo, valores Kd). La diferencia entre dichos dos valores es, por ejemplo, inferior al 50% aproximadamente, al 40% aproximadamente, al 30% aproximadamente, al 20% aproximadamente y/o al 10% aproximadamente como función del valor para la molécula de control/referencia.

[0139] Las frases "sustancialmente reducido(a)", "sustancialmente diferente", tal y como se usan en la presente memoria descriptiva, indican un grado suficientemente elevado de diferencia entre dos valores numéricos (por lo general, uno asociado a una molécula y el otro asociado a una molécula de control/referencia), de forma que alguien experto en la materia consideraría que la diferencia entre los dos valores tiene una significación estadística dentro del contexto de la característica biológica medida por dichos valores (por ejemplo, valores Kd). La diferencia entre dichos dos valores es, por ejemplo, superior al 10% aproximadamente, al 20% aproximadamente, al 30% aproximadamente, al 40% aproximadamente y/o al 50% aproximadamente como función del valor para la molécula de control/referencia.

[0140] En la presente memoria descriptiva, por "vector" se entiende una molécula de un ácido nucleico capaz de transportar otro ácido nucleico al que se ha unido. Un tipo de vector es un "plásmido", que hace referencia a un ADN circular de doble cadena al que se pueden ligar segmentos adicionales de ADN. Otro tipo de vector es un vector fágico. Otro tipo de vector es un vector vírico, en el que segmentos adicionales de ADN se pueden ligar al genoma vírico. Algunos vectores tienen capacidad de replicación autónoma en una célula hospedera en la que se introducen (por ejemplo, vectores bacterianos que tienen un origen bacteriano de replicación y vectores episomales en mamíferos). Otros vectores (por ejemplo, vectores no episomales en mamíferos) pueden integrarse en el genoma de una célula hospedera al introducirse en ésta y, de este modo, se replican al mismo tiempo que el genoma del hospedero. Asimismo, existen vectores capaces de dirigir la expresión de genes a los que están enlazados por su función. Estos vectores se denominan en la presente memoria descriptiva "vectores de expresión recombinantes" o

simplemente "vectores recombinantes". En general, los vectores de expresión de utilidad en las técnicas de ADN recombinante tienen con frecuencia la forma de plásmidos. En la presente especificación, los términos "plásmido" y "vector" son intercambiables, dado que el plásmido es la forma de vector más frecuentemente usada.

[0141] En la presente memoria descriptiva, "polinucleótido" o "ácido nucleico" son términos que se utilizan indistintamente y se refieren a polímeros de nucleótidos de cualquier longitud e incluyen ADN y ARN. Los nucleótidos pueden ser desoxirribonucleótidos, ribonucleótidos, nucleótidos modificados o bases, y/o sus análogos, o cualquier sustrato que se pueda incorporar a un polímero mediante ADN o ARN polimerasa o mediante una reacción sintética. Un polinucleótido puede incluir nucleótidos modificados tales como nucleótidos metilados y sus análogos. De estar presente, la modificación de la estructura del nucleótido se puede producir antes o después del ensamblaje del polímero. La secuencia de nucleótidos puede interrumpirse por componentes no nucleótidos. Un polinucleótido puede comprender una o más modificaciones realizadas tras síntesis, como conjugación a un marcador. Otros tipos de modificaciones incluyen, por ejemplo, "cofias", sustitución de uno o más de los nucleótidos que se producen de forma natural por un análogo; modificaciones de enlaces internucleótidos, como aquellas en las que intervienen enlaces no cargados (por ejemplo, fosfonatos de metilo, fosfotriésteres, fosfoamidatos, carbamatos, etc.) y enlaces cargados (por ejemplo, fosforotioatos, fosforoditioatos, etc.); las que contienen porciones colgantes, como, por ejemplo, proteínas (por ejemplo, nucleasas, toxinas, anticuerpos, péptidos de señal, poli-L-lisina, etc.); las que contienen intercaladores (por ejemplo, acridina, psoralen, etc.); las que contienen quelantes (por ejemplo, metales, metales radioactivos, boro, metales oxidativos, etc.); las que contienen alquilantes; las que contienen enlaces modificados (por ejemplo, ácidos nucleicos anoméricos alfa, etc.), así como formas no modificadas de polinucleótido(s). Asimismo, cualquiera de los grupos hidroxilo presentes habitualmente en los azúcares puede sustituirse, por ejemplo, por grupos de fosfonatos, grupos fosfatos, protegerse mediante grupos protectores estándar, o activarse para preparar enlaces adicionales a nucleótidos adicionales, o puede conjugarse a soportes sólidos o semisólidos. El OH terminal 5' y 3' puede fosforilarse y sustituirse por aminas o porciones de grupos orgánicos en casquete (capping) de 1 a 20 átomos de carbono. Otros hidroxilos también pueden derivatizarse a grupos protectores estándar. Los polinucleótidos también pueden contener formas análogas de azúcares ribosa o desoxirribosa que son generalmente conocidos en la materia; por ejemplo, 2'-O-metil-, 2'-O-allil, 2'-fluoro- o 2'-azido-ribosa, análogos de azúcares carbocíclicos, azúcares α-anoméricos, azúcares epiméricos como arabinosa, xilosas o lixosas, azúcares piranosas, azúcares furanosas, sedoheptulosas, análogos acíclicos y análogos nucleósidos básicos como metil ribosida. Uno o más enlaces de fosfodiéster pueden sustituirse por otros grupos de enlace alternativos. Estos grupos alternativos de enlace incluye formas de realización en las que el fosfato se sustituye por P(O)S ("tioato"), P(S)S ("ditioato"), "(O)NR_2 ("amidato"), P(O)R, P(O)OR', CO o CH2 ("formacetal"), donde cada R o R' es independientemente H o alquilo sustituido o no sustituido (de 1 °C a

20 °C) que contiene opcionalmente un enlace a éter (-O-), arilo, alquenilo, cicloalquilo, cicloalquenilo o aradilo. No todos los enlaces en un polinucleótido tienen que ser idénticos. La descripción anterior se aplica a todos los polinucleótidos mencionados en la presente memoria descriptiva, incluyendo el ARN y el ADN.

[0142] En la presente memoria descriptiva, "oligonucleótido" se refiere por lo general a polinucleótidos cortos, habitualmente de cadena única y sintéticos que suelen tener una longitud inferior a 200 nucleótidos, aunque no necesariamente. Los términos "oligonucleótido" y "poligonucleótido" no se excluyen mutuamente. La descripción anteriormente incluida sobre polinucleótidos es aplicable igualmente y en su integridad a los oligonucleótidos.

[0143] "El Porcentaje (%) de identificación de la secuencia de aminoácidos" con respecto a una secuencia de polipéptidos de referencia se define como el porcentaje de residuos de aminoácidos en un secuencia candidata que son idénticos a los residuos de aminoácidos en la secuencia polipeptídica de referencia, después de alinear las secuencias e introducir espacios, si fuera necesario, para lograr el máximo porcentaje de identificación de la secuencia y no considerar ninguna sustitución conservadora parte de la identificación de la secuencia. A efectos de determinar el porcentaje de identificación de la secuencia de aminoácidos, la alineación se puede conseguir de varias formas que son conocidas en la materia; por ejemplo, usar software comercializado como BLAST, BLAST-2, ALIGN o Megalign (DNASTAR). Los expertos en la materia pueden determinar parámetros apropiados para la alineación de secuencias, incluyendo los algoritmos necesarios para lograr la alineación máxima sobre la longitud completa de las secuencias que se comparan. Sin embargo, a efectos de la presente memoria descriptiva, los valores porcentuales de identificación de la secuencia de aminoácidos se generan utilizando el software de comparación de secuencias ALIGN-2. Dicho software fue creado por Genentech, Inc. y el código fuente ha sido presentado, junto con la documentación del usuario, en la Oficina de Derechos de Autor de Estados Unidos, Washington D.C., 20559, donde está inscrito con el n.º de Registro de Derechos de Autor de Estados Unidos TXU510087. El programa ALIGN-2 está comercializado por Genentech, Inc., South San Francisco, California, o se puede compilar a partir del código fuente. Este programa debe compilarse para su uso en un sistema operativo UNIX, preferentemente UNIX V4.0D digital. El programa ALIGN-2 define todos los parámetros de comparación de secuencias, que no varían.

[0144] En situaciones en las que se emplea ALIGN-2 para comparaciones de secuencias de aminoácidos, el porcentaje de identificación de la secuencia de aminoácidos de una determinada secuencia aminoacídica A con respecto a una determinada secuencia aminoacídica B (lo que también se puede expresar diciendo que una determinada secuencia aminoacídica A tiene o contiene un porcentaje concreto de identificación de la secuencia aminoacídica con respecto a una determinada secuencia aminoacídica B) se calcula de la siguiente manera:

100 veces la fracción X/Y

en la que X es la cantidad de residuos de aminoácidos establecidos como coincidencia total por el programa de alineación de secuencias ALIGN-2 en la alineación hecha por ese programa de A y B, y en la que Y es la cantidad total de residuos de aminoácidos en B. Se detectará que, cuando la longitud de la secuencia de aminoácidos A no sea igual a la longitud de la secuencia de aminoácidos B, el porcentaje de identificación de la secuencia de aminoácidos de A con respecto a B no será igual al porcentaje de identificación de la secuencia de aminoácidos de B con respecto a A. A menos que se indique lo contrario, todos los valores del porcentaje de la identidad de la secuencia de aminoácidos usados en la presente memoria descriptiva se obtienen como se han descrito en el párrafo anterior, utilizando el programa informático ALIGN-2.

[0145] En la presente memoria descriptiva, "STEAP-1" se refiere a cualquier STEAP-1 nativo procedente de vertebarados, incluyendo mamíferos como primates (por ejemplo, humanos, monos cinomolgus) y roedores (por ejemplo, ratas y ratones), a menos que se especifique lo contrario. El término engloba STEAP-1 no procesado de "longitud completa", así como cualquier forma de STEAP-1 que derive del procesamiento en la célula. El término también engloba variantes de STEAP-1 presentes naturalmente, por ejemplo, variantes de ayuste, variantes alélicas o isoformas. La secuencia de aminoácidos del STEAP-1 humana se describe en la Figura 1 (identificador de frecuencia nº 1). En una forma de realización, el STEAP-1 se expresa en la superficie celular, como por ejemplo, en la superficie de una célula normal de próstata, pulmón o colon y ha aumentado la expresión en células cancerosas de próstata, pulmón o colon o metástasis de dichas células cancerosas. La Figura 1 también muestra la secuencia de aminoácidos del STEAP-1 de ratones y del mono cinomolgus (identificador de frecuencia nº 2 y 3 respectivamente).

[0146] Los "anticuerpos" (Ab) e "inmunoglobulinas" (Ig) son glicoproteínas que tienen características estructurales similares. Mientras que los anticuerpos presentan especificidad de unión a un antígeno específico, las inmunoglobulinas incluyen tanto anticuerpos como otras moléculas similares a anticuerpos que por lo general no presentan especificidad a antígenos. Por ejemplo, los polipéptidos de la última clase se producen a niveles bajos en el sistema linfático y a niveles mayores por los mielomas.

[0147] Los términos "anticuerpo" e "inmunoglobulina" se usan indistintamente en el sentido más amplio e incluyen anticuerpos monoclonales (por ejemplo, anticuerpos monoclonales intactos o de longitud completa), anticuerpos policlonales, anticuerpos monovalentes, anticuerpos polivalentes, anticuerpos multiespecíficos (por ejemplo, anticuerpos biespecíficos en tanto en cuanto presenten la actividad biológica deseada) y también pueden incluir determinados fragmentos de anticuerpos (tal como se describe en mayor detalle en la presente memoria descriptiva). Un anticuerpo puede ser quimérico, humano, humanizado o con afinidad madurada, o todos los anteriores.

[0148] Por "anticuerpo anti-STEAP-1" o "anticuerpo que se une a STEAP-1" se entenderá un anticuerpo con capacidad de unión a STEAP-1, con suficiente afinidad para que dicho anticuerpo sea útil como agente diagnóstico o terapéutico, o ambos, para actuar contra STEAP-1. Preferiblemente, el alcance de la unión de un anticuerpo anti-STEAP-1 a una proteína no afín no relacionada con STEAP-1 presenta menos de un 10% aproximadamente de unión del anticuerpo a STEAP-1, según medición, por ejemplo, mediante radioinmunoensayo (RIA). En ciertas formas de realización, un anticuerpo que se une a STEAP-1 tiene una constante de disociación (Kd) de $\leq 1 \mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM o $\leq 0{,}1$ nM. En ciertas formas de realización, un anticuerpo anti-STEAP-1 se une a un epítopo de STEAP-1 que se conserva entre los STEAP-1 de distintas especies.

[0149] Por "región variable" o "dominio variable" de un anticuerpo se entienden los dominios amino terminales de la cadena pesada o ligera del anticuerpo. El dominio variable de la cadena pesada se puede referir como "VH". El dominio variable de la cadena ligera se puede referir como "VL". Estos dominios suelen ser las partes más variables de un anticuerpo y contienen los sitios de unión a antígeno.

[0150] El término "variable" se refiere al hecho de que ciertas porciones de los dominios variables difieren ampliamente en sus secuencias de un anticuerpo a otro y se utilizan para la unión y especificidad de cada anticuerpo en particular con relación a su antígeno particular. Sin embargo, la variabilidad no se distribuye uniformemente a través de los dominios variables de los anticuerpos. Se concentra en tres segmentos denominados regiones de determinación de complementariedad (CDR) o regiones hipervariables (HVR) de los dominios variables tanto de la cadena ligera como de la pesada. Las porciones más conservadas de los dominios variables se llaman regiones de estructura (FR). Cada dominio variable de las cadenas pesada y ligera nativas comprende cuatro FR que, en gran parte, adoptan una configuración de hoja-β, conectadas por tres CDR, que crean bucles que conectan la estructura de la hoja-β, y en algunos casos forman parte de ella. Las CDR de cada cadena se mantienen unidas y en estrecha proximidad mediante las regiones FR y, junto a las CDR de la otra cadena, contribuyen a la formación del sitio de unión a antígeno de los anticuerpos (véase Kabat y col., *Sequences of Proteins of Immunological Interest*, Quinta Edición, National Institute of Health, Bethesda, MD (1991)). Los dominios constantes no participan directamente en la unión de un anticuerpo a un antígeno, pero desarrollan diversas funciones efectoras, como la participación del anticuerpo en la citotoxicidad dependiente de anticuerpos.

[0151] Las "cadenas ligeras" de anticuerpos (inmunoglobulinas) de cualquier vertebrado pueden asignarse a una de dos clases claramente diferenciadas, denominadas kappa (κ) y lambda (λ), en funcion de las secuencias de aminoácidos de sus dominios constantes.

[0152] Según las secuencias de aminoácidos de los dominios constantes de sus cadenas pesadas, los anticuerpos (inmunoglobulinas) pueden asignarse a distintas clases. Existen cinco clases principales de inmunoglobulinas: IgA, IgD, IgE, IgG e IgM, y varias de éstas pueden seguir dividiéndose en subclases

(isotipos), por ejemplo, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 e IgA_2. Los dominios constantes de la cadena pesada que corresponden a las distintas clases de inmunoglobulinas se denominan α, δ, ε, γ y μ, respectivamente. Las estructuras de las subunidades y las configuraciones tridimensionales de las distintas clases de inmunoglobulinas son bien conocidas y se describen de forma general, por ejemplo, en Abbas y col., *Cellular and Mol. Immunology*, 4.ª ed. (2000). Un anticuerpo puede formar parte de una molécula de fusión más amplia, formada por asociación covalente o no covalente del anticuerpo a una o más proteínas o péptidos.

[0153] En la presente memoria descriptiva, los términos "anticuerpo de longitud completa", "anticuerpo intacto" y "anticuerpo entero" se usan indistintamente para hacer referencia a un anticuerpo en su forma sustancialmente intacta, no así a fragmentos de anticuerpo definidos a continuación. Estos términos hacen referencia en concreto a un anticuerpo con cadenas pesadas que contiene la región Fc.

[0154] Los "fragmentos de anticuerpos" contienen únicamente una porción de un anticuerpo intacto, en la que la porción conserva al menos una, aunque también puede conservar la totalidad o la mayoría, de las funciones normalmente asociadas a esa porción cuando está presente en un anticuerpo intacto. En una forma de realización, un fragmento de anticuerpo contiene un sitio de unión a antígeno del anticuerpo intacto y, de este modo, conserva la capacidad para unirse al antígeno. En otra forma de realización, un fragmento de anticuerpo, por ejemplo uno que contiene la región Fc, conserva al menos una de las funciones biológicas habitualmente asociadas a la región Fc cuando está presente en un anticuerpo intacto, como la unión a FcRn, la modulación de la semivida del anticuerpo, la función de la ADCC y la unión a complemento. En una forma de realización, un fragmento de anticuerpo es un anticuerpo monovalente que *in vivo* tiene una semivida sustancialmente similar a la de un anticuerpo intacto. Por ejemplo, un fragmento de anticuerpo de este tipo puede contener un brazo de unión a antígeno enlazado a una secuencia Fc capaz de dar estabilidad *in vivo* al fragmento.

[0155] |La digestión con papaína de anticuerpos produce dos fragmentos idénticos de unión a antígeno, llamados fragmentos "Fab", cada uno de ellos con un solo sitio de unión a antígeno, y un fragmento "Fc" residual, cuyo nombre refleja su capacidad para cristalizarse fácilmente. El tratamiento con pepsina produce un fragmento $F(ab')_2$ que tiene dos sitios de combinacion a antígeno y aún es capaz de establecer un enlace cruzado con el antígeno.

[0156] El "Fv" es el fragmento de anticuerpo mínimo que contiene un sitio completo de unión a antígeno. En una forma de realización, una especie Fv de dos cadenas está compuesta por un dímero de un domino variable de cadena pesada y otro de cadena ligera en una estrecha asociación no covalente. En una especie Fv de cadena única (scFv), un dominio variable de cadena pesada y otro de cadena ligera se pueden enlazar covalentemente mediante un enlazador peptídico flexible, de forma que las cadenas pesada y ligera se asocien en una estructura "dimérica" análoga a la presente en una especie Fv de dos cadenas. En esta

configuración, las tres regiones CDR de cada dominio variable interactúan para definir un sitio de unión a antígeno en la superficie del dímero VH-VL. Colectivamente, las seis regiones CDR confieren al anticuerpo una especificidad de unión a antígeno. No obstante, incluso un dominio variable simple (o la mitad de un Fv que comprenda únicamente tres regiones CDR específicas para un antígeno) tiene la capacidad de reconocer un antígeno y unirse a él, aunque con una afinidad inferior a la del sitio de unión completo.

[0157] El fragmento Fab contiene los dominios variables de cadena pesada y ligera y también contiene el dominio constante de la cadena ligera y el primer dominio constante (CH1) de la cadena pesada. Los fragmentos Fab' se diferencian de los Fab por la adición de algunos residuos en el extremo carboxi terminal del dominio CH1 de la cadena pesada, incluyendo una o más cisteínas de la región bisagra de un anticuerpo. Fab'-SH es la denominación que se da en la presente memoria descriptiva al Fab' en el que el(los) residuo(s) de cisteína de los dominios constantes presenta(n) un grupo tiol libre. Los fragmentos $F(ab')_2$ de anticuerpos se produjeron originalmente como pares de fragmentos Fab' con cisteínas bisagra entre ellos. También se conocen otros acoplamientos químicos de fragmentos de anticuerpos.

[0158] Los fragmentos "Fv de cadena única" o "scFv" de un anticuerpo comprenden los dominios VH y VL del anticuerpo presentes en una cadena simple de polipéptidos. Por lo general, el polipéptido scFv comprende también un enlazador de polipéptidos entre los dominios VH y VL que permite que el scFv forme la estructura deseada para la unión a antígeno. Para una revisión de los scFv, véase Pluckthun en *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg y Moore eds., Springer-Verlag, New York, págs. 269-315 (1994).

[0159] El término "diacuerpos" se refiere a pequeños fragmentos de anticuerpos con dos sitios de unión a antígeno que comprenden un dominio variable de cadena pesada (VH) conectado a un dominio variable de cadena ligera (VL) en la misma cadena de polipéptidos (VH-VL). Mediante el uso de un enlazador que sea demasiado corto para permitir el emparejamiento entre los dos dominios de la misma cadena, estos dominios se ven obligados a emparejarse con los dominios complementarios de otra cadena y crear dos sitios de unión a antígeno. Los diacuerpos pueden ser bivalentes o biespecíficos. Los diacuerpos se describen con más detalle, por ejemplo, en la patente europea EP 404.097; el documento WO93/1161; Hudson y col. (2003) *Nat. Med.* 9:129-134; y Hollinger y col., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Los triacuerpos y tetracuerpos también se describen en Hudson y col. (2003) *Nat. Med.* 9:129-134.

[0160] En la presente memoria descriptiva, "anticuerpo monoclonal" se refiere a un anticuerpo obtenido a partir de una población de anticuerpos sustancialmente homogéneos, es decir, que los anticuerpos individuales que forman la población son idénticos, excepto en posibles mutaciones, por ejemplo, mutaciones de formación natural que podrían estar presentes en cantidades insignificantes. Por lo tanto, el modificador "monoclonal" indica el carácter del anticuerpo, que consiste en que no es una mezcla

de anticuerpos discretos. En determinadas formas de realización, dicho anticuerpo monoclonal incluye típicamente un anticuerpo que comprende una secuencia polipeptídica que se une a una diana, en la que la secuencia polipeptídica de unión a dicha diana se obtuvo mediante un procedimiento que incluye la selección de una sola secuencia polipeptídica de unión a la diana a partir de muchas secuencias polipeptídicas. Por ejemplo, el procedimiento de selección puede ser la selección de un solo clon a partir de muchos clones, como un conjunto de clones de hibridoma, clones fágicos o clones de ADN recombinante. Es conveniente saber que una secuencia seleccionada de unión a la diana puede alterarse adicionalmente, por ejemplo, para mejorar la afinidad para la diana, para humanizar la secuencia de unión a la diana, para mejorar su producción en cultivos celulares, para reducir su inmunogenia *in vivo,* para crear un anticuerpo multiespecífico, etc., y que un anticuerpo que comprenda la secuencia de unión a la diana alterada es, además, un anticuerpo monoclonal de esta invención. En contraste con las preparaciones de anticuerpos policlonales que incluyen típicamente diferentes anticuerpos dirigidos contra diferentes determinantes (epítopos), cada anticuerpo monoclonal de una preparación de anticuerpos monoclonales está dirigido contra un solo determinante en un antígeno. Además de su especificidad, las preparaciones de anticuerpos monoclonales tienen la ventaja de que no suelen estar contaminadas por otras inmunoglobulinas.

[0161] El modificador "monoclonal" indica el carácter del anticuerpo, que consiste en que se ha obtenido de una población de anticuerpos sustancialmente homogénea, y no debe interpretarse que sea necesario producir el anticuerpo por algún procedimiento en particular. Por ejemplo, los anticuerpos monoclonales que se utilicen de conformidad con la presente invención pueden obtenerse a partir de diversas técnicas, incluyendo, por ejemplo, el procedimiento del hibridoma (por ejemplo, Kohler y col., *Nature*, 256: 495 (1975); Harlow y col., *Antibodies*: *A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2.ª ed. 1988); Hammerling y col., en: *Monoclonal Antibodies and T-Cell hybridomas* 563-681 (Elsevier, N.Y., 1981)), los métodos del ADN recombinante (véase, por ejemplo, la patente de EE.UU: N.º 4.816.567), las tecnologías de reproducción de imágenes de fagos (véase, por ejemplo, Clackson y col., *Nature*, 352: 624-628 (1991); Marks y col., *J. Mol. Biol.* 222: 581-597 (1992); Sidhu y col., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee y col., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); y Lee y col., *J. Immunol. Methods* 284(1-2): 119-132(2004), y técnicas para producir anticuerpos humanos o semejantes a los humanos en animales que tienen partes o la totalidad de los loci de la inmunoglobulina humana o los genes que codifican las secuencias de la inmunoglobulina humana (véase, por ejemplo, el documento WO98/24893; el documento WO96/34096; el documento WO96/33735; el documento WO91/10741; Jakobovits y col., *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits y col., *Nature* 362: 255-258 (1993); Bruggemann y col., *Year in Immunol.* 7:33 (1993); las patentes de EE.UU. N.º 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; Marks y col., *Bio.Technology* 10: 779-783 (1992); Lonberg y col., *Nature* 368: 856-859 (1994); Morrison,

Nature, 368: 812-813 (1994); Fishwild y col., *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996) y Lonberg y Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0162] En la presente memoria descriptiva, los anticuerpos monoclonales incluyen específicamente los anticuerpos "quiméricos" en los que una parte de la cadena pesada o ligera, o ambas, es idéntica u homóloga a las secuencias correspondientes de anticuerpos obtenidos de una especie en particular o pertenecientes a una clase o subclase de anticuerpos en particular, mientras que el resto de la(s) cadena(s) son idénticas u homólogas a las secuencias correspondientes de anticuerpos obtenidos de otra especie o pertenecientes a otra clase o subclase de anticuerpos, así como fragmentos de dichos anticuerpos, siempre que exhiban la actividad biológica deseada (patente de EE.UU. N.° 4.816.567 y Morrison y col., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

[0163] Las formas "humanizadas" de los anticuerpos no humanos (por ejemplo, murinos) son anticuerpos quiméricos que contienen una secuencia mínima derivada de la inmunoglobulina no humana. En una forma de realización, un anticuerpo humanizado es una inmunoglobulina humana (anticuerpo receptor) en la que los residuos de una región hipervariable del receptor se sustituyen por residuos procedentes de una región hipervariable de una especie no humana (anticuerpo donante), como ratón, rata, conejo o primates no humanos, que tenga la especificidad, afinidad y capacidad deseadas. En algunos casos, los residuos de la región de estructura (FR) de la inmunoglobulina humana se sustituyen por sus correspondientes residuos no humanos. Además, los anticuerpos humanizados pueden comprender residuos que no se encuentren en el anticuerpo receptor o donante. Estas modificaciones se pueden llevar a cabo para refinar aún más el rendimiento del anticuerpo. En general, un anticuerpo humanizado comprenderá sustancialmente todos los dominios variables, o al menos uno, y típicamente dos, en los que todos, o sustancialmente todos, los bucles hipervariables correspondan a los de una inmunoglobulina no humana y todas, o sustancialmente todas, las FR sean las de una secuencia de inmunoglobulina humana. Opcionalmente, el anticuerpo humanizado también comprenderá al menos una porción de una región constante (Fc) de inmunoglobulina, típicamente humana. Para obtener más detalles, véase Jones y col., *Nature*, 321:522-525 (1986), Riechmann y col., *Nature*, 332:323-329 (1988) y Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). Véanse también los siguientes artículos y las referencias citadas en éstos: Vaswani y Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle y Gross, *Curr. Op. Biotech.* 5:428-433 (1994).

[0164] Un "anticuerpo humano" es aquel que contiene una secuencia de aminoácidos que corresponde a la de un anticuerpo producido por un humano y/o que se ha producido utilizando cualquiera de las técnicas para producción de anticuerpos humanos expuestas en la presente memoria descriptiva. Esta definición de un anticuerpo humano excluye específicamente un anticuerpo humanizado que contenga residuos de unión a antígeno no humanos.

[0165] En la presente memoria descriptiva, por "región hipervariable", "HVR" o "HV" se entienden las regiones de un dominio variable de anticuerpo que son hipervariables en su secuencia y/o forman bucles estructuralmente definidos. Por lo general, los anticuerpos contienen seis regiones hipervariables: tres en la VH (H1, H2, H3) y tres en la VL (L1, L2, L3). En anticuerpos nativos, H3 y L3 muestran la máxima diversidad en las seis regiones hipervariables y se cree que H3 en concreto desempeña un papel único en otorgar una buena especificidad a los anticuerpos. Xu y col. (2000) *Immunity* 13:37-45; Johnson y Wu (2003) en *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ). De hecho, los anticuerpos de camélidos que se producen naturalmente y están formados por una cadena pesada son sólo funcionales y estables en ausencia de la cadena ligera. Hamers-Casterman y col. (1993) *Nature* 363:446-448; Sheriff y col. (1996) *Nature Struct. Biol.* 3:733-736.

[0166] En la presente memoria descriptiva se usan y engloban diversas delineaciones de regiones hipervariables. Las regiones de determinación de complementariedad (CDR) de Kabat están basadas en la variabilidad de las secuencias y son las más frecuentemente usadas (Kabat y col., *Sequences of Proteins of Immunological Interest*, 5.ª Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia, en cambio, se refiere a la ubicación de los bucles estructurales (Chothia y Lesk *J. Mol. Biol.* 196:901-917 (1987)). Las regiones hipervariables de AbM representan una solución intermedia entre las CDR de Kabat y los bucles estructurales de Chothia y se utilizan en el software de modelado de anticuerpos AbM de Oxford Molecular. Las regiones hipervariables "de contacto" se basan en un análisis de las estructuras de cristales complejos disponibles. Los residuos procedentes de cada una de estas regiones hipervariables se indican a continuación.

Bucle	Kabat	AbM	Chothia	Contacto	
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L1	L24-L34	L24-L34	L26-L32	L30-L36	
L2	L50-L56	L50-L56	L50-L52	L46-L55	
L3	L89-L97	L89-L97	L91-L96	L89-L96	
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B	(Numeración de Kabat)
H1	H31-H35	H26-H35	H26-H32	H30-H35	(Numeración de Chothia)
H2	H50-H65	H50-H58	H53-H55	H47-H58	
H3	H95-H102	H95-H102	H96-H101	H93-H101	

[0167] Las regiones hipervariables pueden comprender "regiones hipervariables ampliadas" como se indica a continuación: 24-36 ó 24-34 (L1), 46-56 ó 50-56 (L2) y 89-97 ó 89-96 (L3) en la VL y 26-35 ó 26-35A (H1), 50-65 ó 49-65 (H2) y 93-102, 94-102 ó 95-102 (H3) en la VH. Los residuos de dominio variable se numeran según Kabat y col., mencionado anteriormente, para cada una de estas definiciones. Las regiones hipervariables HVR-H1, HVR-H2, y HVR-H3 de los anticuerpos himanizados anti-STEAP-1 120v.24 de la invención son H26-H35A, H49-H6 y H95-H102 según la numeración de Kabat. Las regiones

hipervariables HVR-L1, HVR-L2 y HVR-L3 de los anticuerpos 120v.24 anti-STEAP-1 humanizados de la invención son L24-34, L50-56 y L89-97 utilizando la numeración de Kabat. En esta memoria descriptiva los términos "HVR" y "CDR" se emplean de manera intercambiable.

[0168] Los residuos de una "estructura" o "FR" son aquellos residuos de un dominio variable distintos de los de una región hipervariable, tal como se define en la presente memoria descriptiva.

[0169] El término "numeración de residuos de dominio variable como en Kabat" o "numeración de la posición de los aminoácidos como en Kabat", y las variaciones de éstos, se refiere al sistema de numeración utilizado para los dominios variables de cadena pesada o los dominios variables de cadena ligera de la compilación de anticuerpos en Kabat y col., *Sequences of Proteins of Immunological Interest*, 5ª Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Utilizando este sistema de numeración, la secuencia lineal real de aminoácidos puede contener menos aminoácidos o aminoácidos adicionales que correspondan a un acortamiento de una FR o HVR del domino variable o a una inserción en ellas, respectivamente. Por ejemplo, un dominio variable de cadena pesada puede incluir una única inserción de aminoácidos (residuo 52a según Kabat) después del residuo 52 de H2 y residuos insertados (por ejemplo, residuos 82a, 82b y 82c, etc., según Kabat) después de un residuo 82 de la FR de la cadena pesada. La numeración de Kabat de los residuos puede estar determinada para un anticuerpo dado por una alineación en regiones de homología de la secuencia del anticuerpo con una secuencia "estándar" numerada según Kabat.

[0170] Un anticuerpo con "maduración de afinidad" es aquél con una o más alteraciones en una o más HVR de éste que dan lugar a una mejora de la afinidad del anticuerpo por el antígeno, en comparación con un anticuerpo parental que no incluye dicha(s) alteración(es). En una forma de realización, un anticuerpo con maduración de afinidad presenta afinidades nanomolares e incluso picomolares para el antígeno diana. Los anticuerpos con maduración de afinidad se producen mediante procedimientos bien conocidos en la materia. En Marks y col., *Bio/Technology*, 10:779-783 (1992), se describe la maduración de afinidad mediante la transposiciónn de los dominios VH y VL. La mutagénesis aleatoria de la HVR y/o los residuos estructurales se describen en: Barbas y col. *Proc Nat. Acad. Sci, USA*, 91:3809-3813 (1994), Schier y col., *Gene*, 169:147-155 (1995), Yelton y col., *J. Immunol.*, 155:1994-2004 (1995), Jackson y col., *J. Immunol.*, 154(7):3310-9 (1995) y Hawkins y col., *J. Mol. Biol.*, 226:889-896 (1992).

[0171] Un anticuerpo "de bloqueo" o un anticuerpo "antagonista" es aquel que inhibe o reduce la actividad biológica del antígeno al que se une. Algunos anticuerpos de bloqueo o anticuerpos antagonistas inhiben sustancial o completamente la actividad biológica del antígeno.

[0172] En la presente memoria descriptiva, un "anticuerpo agonista" es un anticuerpo que imita al menos una de las actividades funcionales de un polipéptido de interés.

[0173] Las "funciones efectoras" de un anticuerpo hacen referencia a aquellas actividades biológicas atribuibles a la región Fc (una región Fc de una secuencia nativa o una región Fc variable de una secuencia aminoacídica) de un anticuerpo y pueden variar con el isotipo del anticuerpo. Los ejemplos de funciones efectoras de un anticuerpo incluyen: La unión de C1q y la citotoxicidad dependiente de complemento, la unión de receptores de Fc, la citotoxicidad celular dependiente de anticuerpos (ADCC), la fagocitosis, la regulación a la baja de los receptores en la superficie celular (por ejemplo, el receptor de células B) y la activación de las células B.

[0174] Los términos "receptor de Fc" o "FcR" describen un receptor que se une a la región Fc de un anticuerpo. En algunas formas de realización, un FcR es un FcR nativo humano. En algunas formas de realización, un FcR es aquel que se une a un anticuerpo IgG (un receptor gamma) e incluye las subclases de receptores FcγRI, FcγRII y FcγRIII, incluidas las variantes alélicas y las formas con ayuste alternativo de estos receptores. Los receptores FcγRII incluyen el FcγRIIA (un "receptor activador") y el FcγRIIB (un "receptor inhibidor"), los cuales tienen secuencias de aminoácidos similares que difieren principalmente en sus dominios citoplasmáticos. El receptor activador FcγRIIA contiene en su dominio citoplasmático un motivo activador inmunorreceptor basado en la tirosina (ITAM). El receptor promotor FcγRIIB contiene en su dominio citoplasmático un motivo inhibidor inmunorreceptor basado en la tirosina (ITIM). (Véase Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). En Ravetch y Kinet, *Annu. Rev. Immunol*, 9:457-92 (1991), Capel y col., *Immunomethods*, 4:25-34 (1994) y de Haas y col., *J. Lab. Clin. Med.*, 126:330-41 (1995) también se revisan los FcRs. Otros FcR, incluidos aquellos que se identifiquen en el futuro, se engloban en el térrmino "FcR" empleado en la presente memoria descriptiva.

[0175] El término "receptor Fc" o "FcR" también incluye el receptor neonatal, FcRn, responsable de la transferencia de las IgG maternas al feto (Guyer y col., *J. Immunol.*, 117:587 (1976) y Kim y col., *J. Immunol.*, 24:249 (1994)) y de la regulación de la homeostasis de las inmunoglobulinas. Se conocen los métodos de medición de la unión al FcRn (véase, por ejemplo, Ghetie 1997, Hinton 2004). La unión al FcRn humano *in vivo* y la semivida sérica de los polipéptidos de unión al RcRn humano de alta afinidad pueden analizarse, por ejemplo, en ratones transgénicos o líneas celulares humanas transfectadas que expresan el FcRn humano, o en primates a los que se administran polipéptidos de variante Fc.

[0176] El documento WO00/42072 (Presta) describe variantes de anticuerpos con capacidad de unión a los FcRs mejoradas o disminuidas. El contenido de esta publicación de patente se incorpora específicamente a la presente memoria descriptiva a modo de referencia. Véase, además, Shields y col., *J. Biol. Chem.*, 9(2): 6591-6604 (2001).

[0177] Las "células efectoras humanas" son leucocitos que expresan uno o más FcRs y realizan funciones efectoras. En determinadas formas de realización, las células expresan al menos el FcγRIII y realizan la función o funciones efectoras de ADCC. Ejemplos de leucocitos humanos que median en la

ADCC serían las células mononucleares de la circulación periférica (PBMC), las células asesinas naturales (NK), los monocitos, las células T citotóxicas y los neutrófilos. Las células efectoras se pueden aislar de una fuente nativa, por ejemplo, de la sangre.

[0178] La "citotoxicidad celular dependiente de anticuerpos" o "ADCC" se refiere a una forma de citotoxicidad en la cual una IgG secretada y unida a los receptores Fc (FcR) presentes en determinadas células citotóxicas (por ejemplo, células asesinas naturales (NK), neutrófilos y macrófagos) posibilita que estas células efectoras citotóxicas se unan específicamente a una célula diana que porte un antígeno y, posteriormente, destruyan dicha célula diana con citotoxinas. Las células primarias para mediar en la ADCC, células NK, expresan sólo FcγRIII, mientras que los monocitos expresan FcγRI, FcγRII y FcγRIII. La expresión del FcR en células hematopoyéticas se resume en la Tabla 3 de la página 464 de Ravetch y Kinet, *Annu. Rev. Immunol*, 9:457-92 (1991). A fin de evaluar la actividad de ADCC de una molécula de interés, se puede realizar un ensayo de ADCC *in vitro*, como el que se describe en las patentes de EE.UU. N.º 5.500.362 ó 5.821.337, o se puede realizar el ensayo de Presta que se describe en la patente de EE.UU. N.º 6.737.056. Entre las células efectoras útiles para estos ensayos se encontrarán las células sanguíneas mononucleares periféricas (PBMC) y las asesinas naturales (NK). Como alternativa, o adicionalmente, la actividad de ADCC de la molécula de interés se puede evaluar *in vivo*, por ejemplo, en un modelo animal como el que se desvela en Clynes y col., *PNAS (EE.UU.)* 95:652-656 (1998).

[0179] La "citotoxicidad dependiente de complemento" o "CDC" se refiere a la lisis de una célula diana en presencia del complemento. La vía clásica de activación del complemento se inicia por la unión del primer componente del sistema del complemento (C1q) a los anticuerpos (de la subclase apropiada) que están unidos a su antígeno cognado. A fin de evaluar la activación del complemento, se puede efectuar un ensayo de CDC, como por ejemplo, el que se describe en Gazzano-Santoro y col., *J. Immunol. Methods*, 202:163 (1996).

[0180] Las variantes polipeptídicas con secuencias aminoacídicas de la región Fc alteradas y capacidad aumentada o disminuida de unión a Cq1 se describen en la patente de EE.UU. N.º 6.194.551B1 y el documento WO99/51642. El contenido de dichas publicaciones de patentes se incorpora específicamente en la presente memoria descriptiva a modo de referencia. Véase, además, Idusogie y col., *J. Immunol.* 164: 4178-4184 (2000).

[0181] Por "polipéptido que comprende una región Fc" se entiende un polipéptido, como un anticuerpo o una inmunoadhesina, que comprende una región Fc. La lisina en el extremo C terminal (residuo 447 según el sistema de numeración de EU) de la región Fc puede eliminarse, por ejemplo, durante la purificación del polipéptido o recombinando el ácido nucleico que codifica el polipéptido. Por lo tanto, una composición que contenga un polipéptido con una región Fc según esta invención puede comprender

polipéptidos con el K447, con todos los K447 eliminados, o una mezcla de polipéptidos con el residuo K447 o sin él.

[0182] A efectos de la presente memoria descriptiva, una "estructura humana del aceptor" es una estructura que comprende la secuencia aminoacídica de una estructura de VL o VH derivada de una estructura de inmunoglobulinas humanas, o de una estructura de consenso humana. Una estructura humana del aceptor "derivada de" una estructura de inmunoglobulinas humanas o una estructura de consenso humana puede comprender la misma secuencia de aminoácidos de éstas o puede contener cambios preexistentes en la secuencia de aminoácidos. En algunas formas de realización, el número de cambios preexistentes en la secuencia de aminoácidos es 10 o menos, 9 o menos, 8 o menos, 7 o menos, 6 o menos, 5 o menos, 4 o menos, 3 o menos o 2 o menos. En el caso de que haya cambios preexistentes de aminoácidos en una VH, estos cambios, preferentemente, se dan sólo en tres, dos o una de las posiciones 71H, 73H y 78H; por ejemplo, los residuos de aminoácidos en estas posiciones pueden ser 71A, 73T y/o 78A. En una forma de realización, la estructura humana de VL del aceptor tiene una secuencia idéntica a la de la estructura de VL de la inmunoglobulina humana o a la estructura de consenso humana.

[0183] Una "estructura de consenso humana" es una estructura que representa los residuos de aminoácidos más frecuentes en una selección de secuencias de estructura de VL o VH de inmunoglobulina humana. Por lo general, la selección de secuencias de VL o VH de inmunoglobulina humana se realiza en un subgrupo de secuencias de dominio variable. Por lo general, el subgrupo de secuencias es un subgrupo como en Kabat y col., *Sequences of Proteins of Immunological Interest*, 5.ª Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). En una forma de realización, para VL, el subgrupo es un subgrupo kappa I como en Kabat y col., mencionado anteriormente. En una forma de realización, para VH, el subgrupo es un subgrupo III como en Kabat y col., mencionado anteriormente.

[0184] Una "estructura de consenso del subgrupo III de VH" comprende la secuencia de consenso obtenida de las secuencias de aminoácidos en el subgrupo III variable pesado de Kabat y col., mencionado anteriormente. En una forma de realización, la secuencia de aminoácidos de la estructura de consenso del subgrupo III de VH comprende al menos una parte, o la totalidad, de las siguientes secuencias: EVQLVESGGGLVQPGGSLRLSCAAS (FR-H1, identificador de secuencia n.º: 21)-HVR-H1-WVRQAPGKGLEWV (FR-H2, identificador de secuencia n.º: 22)-HVR-H2-RFTISRDTSKNTLYLQMNSLRAEDTAVYYCAR (FR-H3, identificador de secuencia n.º: 23)-HVR-H3-WGQGTLVTVSS (FR-H4, identificador de secuencia n.º: 24).

[0185] Una "estructura de consenso del subgrupo I de VL" comprende la secuencia de consenso obtenida de las secuencias de aminoácidos en el subgrupo I kappa variable ligero de Kabat y col., mencionado anteriormente. En una forma de realización, la secuencia de aminoácidos de la estructura de consenso del subgrupo I de VH comprende al menos una parte, o la totalidad, de las siguientes secuencias:

DIQMTQSPSSLSASVGDRVTITC (FR-L1, identificador de secuencia n.º: 17)-HVR-L1-WYQQKPGKAPKLLIY (FR-L2, identificador de secuencia n.º: 18)-HVR-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, identificador de secuencia n.º: 19)-HVR-L3-FGQGTKVEIKR (FR-L4, identificador de secuencia n.º: 20). Una "secuencia de señal de la secreción" o "secuencia de señal" se refiere a la secuencia de ácido nucleico que codifica un péptido de señal corta que puede utilizarse para dirigir una proteina de interés recientemente sintetizada a través de una membrana celular, generalmente la membrana interna o ambas membranas, tanto la interna como la externa, de las procariotas. Como tal, la proteína de interés, como por ejemplo el polipéptido de cadena pesada o ligera de inmunoglobulina, se secreta en un periplasma de las células hospederas procarióticas o en el medio de cultivo. El péptido de señal que se codifica mediante la secuencia de señal de la secreción puede ser endógeno o exógeno para las células hospederas, incluyendo los péptidos de señal propios de los polipéptidos que se expresarán. Las secuencias de señal de la secreción generalmente se encuentran presentes en el extremo amino de un polipéptido que se expresará, y por lo general se eliminan enzimáticamente del citoplasma entre la biosíntesis y la secreción de un polipéptido. Por lo tanto, el péptido de señal normalmente no se encuentra presente en una proteína madura.

[0186] Un "aminoácido de cisteína libre" se refiere a un residuo de aminoácido cisteína diseñado para obtener un anticuerpo progenitor que tiene un grupo funcional tiol (-SH) y no está acoplado a, o a una parte de, un puente disulfuro intramolecular o intermolecular.

[0187] El término "valor de reactividad del tiol" es una caracterización cuantitativa de la reactividad de los aminoácidos de cisterna libre. El valor de reactividad del tiol es el porcentaje de un aminoácido de cisteína presente en un anticuerpo diseñado con cisteína que reacciona con un reactivo tiol-específico y convertido a un valor máximo de 1. Por ejemplo, un aminoácido de cisteína libre en un anticuerpo diseñado con cisteína que reacciona en 100% de producto con un reactivo tiol-específico, como por ejemplo un reactivo biotina-maleimida, para formar un anticuerpo denominado biotina tiene un valor de reactividad del tiol de 1,0. Otro aminoácido cisteína diseñado en el mismo o en otro anticuerpo progenitor que reacciona en 80% de producto con un reactivo tiol-específico tiene un valor de reactividad del tiol de 0,8. Otro aminoácido cisteína diseñado en el mismo o en otro anticuerpo progenitor que no logra en absoluto reaccionar con un reactivo tiol-específico tiene un valor de reactividad del tiol de 0. La determinación del valor de reactividad del tiol de una cisteína específica puede realizarse mediante ensayo ELISA, espectroscopía de masas, cromatografía líquida, autorradiografía u otras pruebas analíticas cuantitativas. Entre los reactivos tiol-específicos que permiten la captura del anticuerpo diseñado con cisteína y la comparación y cuantificación de la reactividad de la cisteína se incluyen biotina-PEO-maleimida ((+)-biotinil-3-maleimidopropionamidil-3,6-dioxaoctainodiamina, Oda et al (2001) *Nature Biotechnology* 19:379-382 (Pierce Biotechnology, Inc.), Biotina-BMCC, PEO-Yodoacetyl Biotina,

Yodoacetil-LC-Biotina y Biotina-HPDP (Pierce Biotechnology, Inc.), y Nα-(3-maleimidilpropionil)biocitina (MPB, Molecular Probes, Eugene, OR). Otras fuentes comerciales para la obtención de reactivos de ligandos de biotinilación, bifuncionales o multifuncionales incluyen a Molecular Probes, Eugene, OR y Sigma, St. Louis, MO.

[0188] Un "anticuerpo progenitor" es un anticuerpo que comprende una secuencia de aminoácido a partir de la cual uno o más residuos de aminoácido son sustituidos por uno o más residuos de cisteína. El anticuerpo progenitor puede comprender una secuencia nativa o natural. El anticuerpo progenitor puede tener modificaciones de la secuencia de aminoácido preexistente (como por ejemplo adiciones, eliminaciones y/o sustituciones) relacionadas con otro tipo nativo, natural o formas modificadas de un anticuerpo. Un anticuerpo progenitor puede dirigirse contra un antígeno objetivo de interés, p.ej. un polipéptido biológicamente importante. También se contemplan los anticuerpos dirigidos contra antígenos no polipéptidos (como los antígenos glicolípidos asociados a un tumor; véase 5091178 de EE.UU.).

[0189] Por "afinidad de unión" generalmente se entiende la fuerza de la suma total de las interacciones no covalentes entre un único sitio de unión de una molécula (por ejemplo, un anticuerpo) y su molécula de enlace (por ejemplo un antígeno). A menos que se especifique lo contrario, tal como se usa en la presente memoria descriptiva, por "afinidad de unión" se entiende la afinidad de unión intrínseca que refleja una interacción 1:1 entre miembros de un par de unión (por ejemplo, anticuerpo y antígeno). La afinidad de una molécula X por su molécula de enlace Y se puede representar por lo general mediante la constante de disociación (Kd). La afinidad se puede medir mediante métodos habituales conocidos en la especialidad, incluidos los descritos en la presente memoria descriptiva. Los anticuerpos de baja afinidad suelen unirse al antígeno lentamente y suelen disociarse de inmediato, mientras que los anticuerpos de alta afinidad por lo general se unen al antígeno más rápidamente y suelen permanecer unidos durante más tiempo. Existen diversos métodos para medir la afinidad de unión conocidos en la especialidad y todos ellos se pueden utilizar para los fines de la presente invención. A continuación, se describen, a modo de ejemplo, formas de realización específicas.

[0190] En una forma de realización, el "Kd" o el "valor Kd" según esta invención se mide mediante un ensayo de unión a antígeno radiomarcado (RIA) realizado con la versión Fab de un anticuerpo de interés y su antígeno, como se describe en el siguiente ensayo. La afinidad de unión en solución de Fab a antígenos se mide equilibrando los Fab con una concentración mínima del antígeno marcado (^{125}I) en presencia de una serie de titulación de antígeno no marcado y, después, capturando el antígeno unido con una placa de anticuerpo de revestimiento anti-Fab (Chen, y col., (1999) *J. Mol Biol* 293:865-881). Para establecer las condiciones del ensayo, las placas de microtitulación (Dynex) se recubren durante toda la noche con 5 μg/ml de un anticuerpo anti-Fab de captura (Cappel Labs) en 50 mM de carbonato sódico (pH 9,6) y, a continuación, se bloquean con 2% (p/v) de albúmina de suero bovino en PBS durante dos a cinco

horas a temperatura ambiente (23 °C aproximadamente). En una placa no adsorbente (Nunc n.° 269620), 100 pM o 26 pM de antígeno marcado con [^{125}I] se mezclan con diluciones seriadas de un Fab de interés (por ejemplo, que concuerde con la evaluación del anticuerpo anti-VEGF, Fab-12, en Presta y col., (1997) *Cancer Res.* 57:4593-4599). A continuación, el Fab de interés se incuba durante toda la noche; no obstante, la incubación puede continuar durante más tiempo (por ejemplo, aproximadamente 65 horas) para garantizar que se alcance el equilibrio. Después, las mezclas se transfieren a la placa de captura para su incubación a temperatura ambiente (por ejemplo, durante una hora). A continuación, se extrae la solución y se lava la placa ocho veces con Tween-20 al 0,1% en PBS. Una vez secas las placas, se añaden 150 µl/pocillo de centelleador (MicroScint-20; Packard) y las placas se cuentan en un contador gamma Topcount (Packard) durante diez minutos. Se eligen concentraciones de cada Fab que proporcionen menos o igual del 20% de unión máxima para utilizarlas en ensayos de unión competitivos.

[0191] Según otra forma de realización, el Kd o valor Kd se mide utilizando análisis de resonancia plasmón de superficie que usa BIAcore™-2000 o BIAcore™-3000 (BIAcore, Inc., Piscataway, New Jersey) a 25 °C con chips CM5 de antígeno inmovilizado a ~10 unidades de respuesta (RU). Los biosensores de dextrano carboximetilado (CM5, BIAcore® Inc) se activan con clorhidrato de N-etil-N'-(3-dimetilaminopropil)-carbodiimida (EDC) y N-hidroxisuccinimida (NHS), según las instrucciones del proveedor. El antígeno se diluye con 10 mM de acetato sódico, pH 4,8, a 5 µg/ml (~0,2 µM) antes de la inyección a un caudal de 5 µl/minuto para lograr aproximadamente 10 unidades de respuesta (RU) de la proteína emparejada. Tras la inyección del antígeno, se inyecta 1 M de etanolamina para bloquear grupos no reaccionados. Para mediciones cinéticas, se inyecta el doble de diluciones seriadas de Fab (0,78 nM a 500 mM) en PBS con 0,05% de Tween 20 (PBST) a 25 °C a un caudal de 25 µl/min aproximadamente. Las tasas de asociación (k_{on}) y las tasas de disociación (k_{off}) se calculan utilizando un sencillo modelo de unión uno a uno de Langmuir (BIAcore® Evaluation Software, versión 3.2) al encajar simultáneamente los sensogramas de asociación y disociación. La constante de disociación de equilibrio (Kd) se calcula como el cociente k_{off}/k_{on}. Véase, por ejemplo, Chen, Y., y col., (1999) *J. Mol Biol* 293:865-881. Si la tasa de asociación excede de 10^6 M^{-1} s^{-1} según el ensayo de resonancia plasmón de superficie mencionado anteriormente, la tasa de asociación se puede determinar utilizando una técnica de absorción fluorescente que mide el aumento o disminución de la intensidad de la emisión de fluorescencia (excitación = 295 nm; emisión = 340 nm, 16 nm de frecuencia) a 25 °C de 20 nM de un anticuerpo anti-antígeno (forma Fab) en PBS, pH 7,2, en presencia de concentraciones cada vez más elevadas de antígeno según medición en un espectrómetro, como un espectrómetro equipado con módulo "stop-flow" (interruptor de flujo) (Aviv Instruments) o un espectrómetro SLM-Aminco serie 8000 (ThermoSpectronic) con una cubeta con agitador.

[0192] También se puede determinar una "tasa de asociación" o "k_{on}" según esta invención como se describe anteriormente, utilizando un sistema BIAcore™-2000 o un sistema BIAcore™-3000 (BIAcore®, Inc., Piscataway, NJ).

[0193] Un "trastorno" es un proceso o enfermedad que se beneficiaría del tratamiento con una sustancia/molécula o método de la invención. Esto implica trastornos crónicos y agudos, incluidos los procesos patológicos que predisponen al mamífero al trastorno en cuestión. Algunos ejemplos, entre otros, de los trastornos que se tratarán en la presente memoria descriptiva incluyen condiciones cancerosas tales como cánceres o metástasis de próstata, pulmón y colon.

[0194] Los términos "trastorno de la proliferación celular" y "trastorno de la proliferación" se refieren a trastornos asociados a algún grado de proliferación celular anómala. En una forma de realización, el trastorno de la proliferación celular es un cáncer.

[0195] En la presente memoria descriptiva, por "tumor" se entiende todo crecimiento y proliferación celular neoplásica, tanto benigna como maligna, así como todas las células y tejidos cancerosos y precancerosos. Tal y como se usan en la presente memoria descriptiva, los términos "cáncer", "cancerígeno", "trastorno de proliferación celular", "trastorno de proliferación" y "tumor" no se excluyen mutuamente.

[0196] Los términos "cáncer" y "cancerígeno" describen o se refieren al estado fisiológico de los mamíferos típicamente caracterizado por un crecimiento celular no regulado. Los ejemplos de cáncer incluyen, entre otros, carcinoma, linfoma, blastoma, sarcoma y leucemia o tumores linfoides. Ejemplos más concretos de estas clases de cáncer son el cáncer de células escamosas (por ejemplo, el cáncer de células escamosas epiteliales), cáncer de pulmón incluyendo el cáncer de células pulmonares pequeñas, el adenocarcinoma pulmonar y el carcinoma pulmonar escamoso, el cáncer de peritoneo, el cáncer hepatocelular, el cáncer gástrico o estomacal (incluyendo el cáncer gastrointestinal), el cáncer pancreático, el glioblastoma, el cáncer de cuello uterino, el cáncer de ovario, el cáncer de hígado, el cáncer de vejiga, cáncer de tracto urinario, el hepatoma, el cáncer de mama, el cáncer de colon, el cáncer rectal, el cáncer colorrectal, el carcinoma endometrial o uterino, el carcinoma de las glándulas salivales, el cáncer de riñón o renal, el cáncer de próstata, el cáncer vulval, el cáncer de tiroides, el carcinoma hepático, el carcinoma anal, el carcinoma de pene, el melanoma, el mieloma múltiple y el linfoma de células B, el cáncer de cerebro, así como el de cabeza y cuello, y metástasis asociadas.

[0197] Una "célula que expresa a STEAP-1" es una célula que expresa a la STEAP-1 endógena o transfectada en la superficie celular. Un "cáncer que expresa a STEAP-1" es un cáncer que comprende células con presencia de la proteína STEAP-1 en la superficie celular. Un "cáncer que expresa a STEAP-1" produce niveles suficientes de STEAP-1 en la superficie de sus células, de modo que puede unírsele un anticuerpo anti-STEAP-1 y tener un efecto terapéutico sobre dicho cáncer. Un cáncer que "sobreexpresa" a

STEAP-1 es aquél que tiene niveles significativamente superiores de STEAP-1 en la superficie de sus células en comparación con una célula no cancerosa de la misma clase de tejido. Dicha sobreexpresión puede estar causada por una amplificación génica o por un aumento de la transcripción o traducción. La sobreexpresión de STEAP-1 puede determinarse en un ensayo de diagnóstico o prognosis evaluando el aumento de los niveles de STEAP-1 presentes en la superficie de una célula (por ejemplo, mediante un ensayo inmunohistoquímico; análisis FACS). Como alternativa, o adicionalmente, se pueden medir los niveles de ácido nucleico o de ARNm que codifica a STEAP-1 presentes en la célula, p.ej. a través de la hibridación *in situ* con fluorescencia (FISH, véase el documento WO98/45479 publicado en octubre de 1998), las técnicas de Southern o Northern, o las técnicas de reacción en cadena de la polimerasa (PCR), como la PCR cuantitativa en tiempo real (RT-PCR). También se puede estudiar la sobreexpresión de STEAP-1 midiendo el antígeno vertido en un fluido biológico como el suero, por ejemplo, utilizando ensayos basados en anticuerpos (véase también la patente de EE.UU. nº 4.933.294 concedida el 12 de junio de 1990, el documento WO91/05264 publicado el 18 de abril de 1991, la patente de EE.UU. nº 5.401.638 concedida el 28 de marzo de 1995 y Sias y col., *J. Immunol. Methods*, 132: 73-80 (1990)). Aparte de los anteriores ensayos, el experto en la materia también dispone de diversos ensayos *in vivo*. Por ejemplo, se puede exponer células del cuerpo del paciente a un anticuerpo que esté opcionalmente etiquetado con una etiqueta detectable, por ejemplo, un isótopo radiactivo, y evaluar la unión del anticuerpo a dichas células, por ejemplo, mediante la exploración externa por radiactividad o analizando una biopsia tomada de un paciente previamente expuesto al anticuerpo. Entre los tipos de cáncer que expresan a STEAP-1 se incluyen el cáncer de próstata, el de pulmón y el de colon.

[0198] En la presente memoria descriptiva, "tratamiento" (y variaciones de dicho término, como "tratar" o "tratados") se refiere una intervención clínica en un intento de alterar el proceso natural del individuo o célula tratada y se puede realizar como prevención o durante la evolución de la enfermedad. Son efectos deseables del tratamiento la prevención de la enfermedad o de su recidiva, el alivio de los síntomas, la disminución de cualquier consecuencia patológica directa o indirecta de la enfermedad, la prevención de la metástasis, la disminución del ritmo de progresión de la enfermedad, la mejora o los cuidados paliativos del estado de la enfermedad y la remisión o un pronóstico de mejoría. En algunas formas de realización, los anticuerpos de la invención se utilizan para retrasar el desarrollo de una enfermedad o trastorno o para ralentizar la progresión de una enfermedad o trastorno.

[0199] Los parámetros anteriores para evaluar el éxito del tratamiento y la mejora de la enfermedad se pueden medir fácilmente mediante procedimientos habituales conocidos por un médico. En el caso del tratamiento del cáncer, la eficacia se puede medir, por ejemplo, evaluando el tiempo hasta la progresión de la enfermedad (TTP) y/o determinando la tasa de respuesta (RR). En el caso del cáncer del próstata, el progreso de la terapia puede evaluarse mediante métodos de rutina, por lo general midiendo los

niveles de PSA (antígeno específico de la próstata); cuanto mayor es el nivel de PSA en la sangre, más extendido está el cáncer. Se dispone de ensayos comerciales para la detección de PSA, p.ej., los kits de ensayo Tandem-E y Tandem-R de Hybitech, el ensayo policlonal ProsCheck de Yang (Yang Labs, Bellevue, WA); Imx de Abbott (Abbott Labs, Abbott Park, IL), etc. La metástasis puede determinarse mediante estudios de diseminación y exploración ósea y pruebas para medir el nivel de calcio y otras enzimas a fin de determinar si el cáncer se ha extendido hasta los huesos. También se puede realizar una TAC para detectar la extensión por la pelvis y los nódulos linfáticos de la zona. Se realizan estudios radiológicos de tórax y mediciones, mediante métodos conocidos, de los niveles enzimáticos hepáticos para detectar metástasis en los pulmones y el hígado, respectivamente. Otros métodos habituales para realizar un seguimiento de la enfermedad incluyen ecografía transrectal (TRUS) y biopsia transrectal por punción con aguja gruesa (TRNB).

[0200] Un "sujeto" es un vertebrado. En algunas formas de realización, el vertebrado es un mamífero. Los mamíferos incluyen animales de granja (como vacas), animales utilizados en actividades deportivas, animales de compañía (como gatos, perros y caballos), primates, ratones y ratas. En algunas formas de realización, el mamífero es un humano.

[0201] Una "cantidad eficaz" es una cantidad eficaz, a las dosis y durante el tiempo necesarios para lograr el efecto terapéutico o profiláctico deseado. Una "cantidad terapéuticamente eficaz" de una sustancia/molécula de la invención puede variar según factores como el estado de la enfermedad, la edad, el sexo y el peso del individuo y la capacidad de la sustancia/molécula para dar lugar a una respuesta deseada en el individuo. Una cantidad terapéuticamente eficaz comprende aquella cantidad a la que los efectos tóxicos o perjudiciales de la sustancia/molécula son contrarrestados por los efectos terapéuticamente beneficiosos. Una "cantidad eficaz desde el punto de vista profiláctico" es una cantidad eficaz, a las dosis y durante el tiempo necesarios, para lograr el efecto terapéutico o profiláctico deseado. Habitualmente, aunque no necesariamente, dado que una dosis profiláctica se utiliza en sujetos antes de la enfermedad o en una etapa temprana, la cantidad profilácticamente eficaz sería inferior a la cantidad terapéuticamente eficaz. En el caso del cáncer, la cantidad terapéuticamente efectiva del fármaco puede reducir el número de células cancerosas y el tamaño del tumor; inhibir (es decir, lentificar hasta cierto punto y preferentemente detener) la infiltración de células cancerosas en los órganos periféricos; inhibir (es decir, lentificar hasta cierto punto y preferiblemente detener) la metástasis tumoral; inhibir, hasta cierto punto, el crecimiento tumoral; y/o aliviar hasta cierto punto uno o más de los síntomas asociados al cáncer. Véase la anterior definición de "tratamiento". Con relación a la capacidad del fármaco para evitar el crecimiento de las células cancerosas, y/o eliminar las existentes, éste puede ser citostático y/o citotóxico.

[0202] La administración "crónica" se refiere a la administración del agente o agentes de un modo continuo, frente a un modo agudo, con el fin de mantener el efecto terapéutico inicial (actividad) durante un período prolongado de tiempo. La administración "intermitente" es el tratamiento que no se realiza de forma consecutiva sin interrupción, sino que tiene carácter cíclico.

[0203] La administración "en combinación con" uno o más agentes terapéuticos adicionales incluye la administración simultánea (concurrente) y consecutiva en cualquier orden.

[0204] Los "vehículos", tal como se utiliza este término en la presente memoria descriptiva, incluyen vehículos, excipientes o estabilizadores farmacéuticamente aceptables que no sean tóxicos para la célula o el mamífero expuesto a los mismos con las dosis y concentraciones empleadas. Con frecuencia, el vehículo fisiológicamente aceptable es una solución acuosa de pH tamponado. Entre los ejemplos de vehículos fisiológicamente aceptables están tampones como fosfato, citrato y otros ácidos orgánicos; antioxidantes como ácido ascórbico; un polipéptido de bajo peso molecular (inferior a 10 residuos); proteínas, como albúmina sérica, gelatina o inmunoglobulinas; polímeros hidrófilos, como polivinilpirrolidona; aminoácidos como glicina, glutamina, asparragina, arginina o lisina; monosacáridos, disacáridos y otros carbohidratos, como glucosa, manosa o dextrinas; agentes quelantes como EDTA; alcoholes azucarados, como manitol o sorbitol; contraiones formadores de sales, como el sodio; y/o surfactantes no iónicos, como TWEEN™, polietilenglicol (PEG) y PLURONICS™.

[0205] "Marcador" tal como se utiliza en la presente memoria descriptiva se refiere a un compuesto o a una composición detectable que se conjuga directa o indirectamente con el anticuerpo de manera de generar un anticuerpo "marcado". El marcador puede ser detectable por sí mismo (por ejemplo, marcadores de isótopos radioactivos o fluorescentes) o, en el caso de un marcador enzimático, puede catalizar la alteración química de un compuesto o de una composición sustrato que sea detectable.

[0206] El término "señalizado con epítopo" utilizado en la presente memoria descriptiva se refiere a un polipéptido quimérico que contiene un polipéptido anticuerpo anti-PSCA fusionado a un "polipéptido señalizado". El polipéptido señalizado tiene suficientes residuos para proporcionar un epítopo contra el cual se pueda elaborar un anticuerpo, pero es suficientemente corto para que no interfiera en la actividad del polipéptido Ig al cual se fusiona. El polipéptido señalizado es también preferentemente único, a fin de que el anticuerpo no tenga una reacción cruzada sustancial con otros epítopos. Por lo general, los polipéptidos señalizados aptos tienen al menos 6 residuos de aminoácidos, siendo la cantidad habitual entre 8 y 50 (preferentemente, entre 10 y 20).

[0207] Según su definición en la presente memoria descriptiva, una "molécula pequeña" tiene un peso molecular inferior a unos 500 daltons.

[0208] El término "prospecto adjunto" se utiliza para hacer referencia a las instrucciones habitualmente incluidas en los envases comerciales de los productos terapéuticos, las cuales contienen información sobre las indicaciones, el uso, la posología, la administración, las contraindicaciones y/o las advertencias de uso de dichos productos terapéuticos.

[0209] Un "ácido nucleico aislado" es un ácido nucleico, p.ej., un ARN, ADN o un polímero combinado que es sustancialmente separado de otras secuencias del ADN del genoma, así como proteínas o complejos como los ribosomas y las polimerasas, que acompañan naturalmente a una secuencia nativa. El término comprende a una secuencia de ácido nucleico que ha sido extraída del medio en el que se genera naturalmente, e incluye fragmentos aislados de ADN recombinantes o clonados y análogos químicamente sintetizados o análogos sintetizados biológicamente mediante sistemas heterólogos. Una molécula sustancialmente pura incluye formas aisladas de la molécula.

[0210] Un "vector" incluye vectores lanzadera y de expresión. Por lo general, el constructo del plásmido también incluirá un origen de replicación (por ejemplo, el origen ColE1 de replicación) y un marcador seleccionable (por ejemplo, resistencia a tetraciclina o ampicilina), para replicación y selección, respectivamente, de los plásmidos en bacterias. Un "vector de expresión" se refiere a un vector que contiene las secuencias de control o elementos reguladores necesarios para la expresión de los anticuerpos, incluido el fragmento del anticuerpo de la invención, en células eucarióticas o bacterianas. Los vectores aptos se dan a conocer más adelante.

[0211] La célula que produce un anticuerpo anti-STEAP-1 de la invención incluirá la célula del hibridoma progenitor, p.ej. los hibridomas que se depositan con el ATCC, así como las células hospederas bacterianas y eucarióticas en las que se ha introducido el ácido nucleico que codifica a los anticuerpos. Las células hospederas aptas se dan a conocer más adelante.

[0212] Un "agente inhibidor del crecimiento" se refiere, en el contexto de la presente memoria descriptiva, a un compuesto o composición que inhibe el crecimiento de una célula, especialmente una célula cancerosa que exprese PSCA, tanto *in vitro* como *in vivo*. Así, el agente inhibidor del crecimiento puede reducir significativamente el porcentaje de células que expresen PSCA en la fase S. Ejemplos de agentes inhibidores del crecimiento serían los agentes que bloquean la progresión del ciclo celular (en un punto distinto de la fase S), como los agentes que inducen la detención de la G1 y la fase M. Entre los bloqueadores clásicos de la fase M se incluyen las vincas (vincristina y vinblastina), los taxanos y los

inhibidores de la topoisomerasa II como la doxorrubicina, la epirrubicina, la daunorrubicina, el etopósido y la bleomicina. Esos agentes que detienen la G1 también se desbordan en la detención de la fase S, por ejemplo, los agentes de alquilación del ADN como el tamoxifeno, la prednisona, la dacarbacina, la mecloretamina, el cisplatino, el metotrexato, el 5-fluorouracilo y la ara-C. Puede encontrarse más información al respecto en *The Molecular Basis of Cancer*, Mendelsohn y Israel, eds., capítulo 1, titulado "Cell cycle regulation, oncogenes, and antineoplastic drugs" (Regulación del ciclo celular, oncogenes y fármacos antineoplásicos) por Murakami y col. (WB Saunders: Filadelfia, 1995), especialmente en la página 13. Los taxanos (paclitaxel y docetaxel) son medicamentos anticancerígenos derivados ambos del tejo. El docetaxel (TAXOTERE®, Rhone-Poulenc Rorer) se deriva del tejo europeo y es un análogo semisintético del paclitaxel (TAXOL®, Bristol-Myers Squibb). El paclitaxel y el docetaxel promueven la unión de microtúbulos a partir de dímeros de tubulina y estabilizan los microtúbulos al evitar la despolimerización, que ocasiona la inhibición de mitosis en células.

[0213] El término "agente citotóxico", en el contexto de la presente memoria descriptiva, se refiere a una sustancia que inhibe o impide la función de las células y/o causa muerte o destrucción celular. El término pretende incluir isótopos radioactivos (por ejemplo, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} e isótopos radiactivos de Lu), agentes quimioterapéuticos (por ejemplo, metotrexato, adriamicina, alcaloides de la vinca (vincristina, vinblastina, etoposido), doxorubicina, melfalan, mitomicina C, clorambucil, daunorubicina u otros agentes intercaladores, enzimas y fragmentos de éstas como enzimas nucleolíticas, antibióticos y toxinas como toxinas de molécula pequeña o toxinas enzimáticamente activas de origen bacteriano, fúngico, vegetal o animal, incluidos fragmentos y/o variantes de éstos, toxinas, agentes inhibidores del crecimiento, fracciones de fármacos, y los diversos agentes antineoplásicos o antitumorales divulgados más adelante. Otros agentes citotóxicos se describen más adelante. Un agente tumoricida ocasiona la destrucción de las células tumorales.

[0214] Una "toxina" es una sustancia con capacidad para tener un efecto perjudicial en el crecimiento o proliferación de una célula.

[0215] Un "agente quimioterapéutico" es un compuesto químico útil en el tratamiento del cáncer. Ejemplos de agentes quimioterapéuticos incluyen agentes alquilantes como la tiotepa y la ciclofosfamida (CYTOXAN®); los sulfonatos de alquilo como el busulfán, el improsulfán y el piposulfán; las aziridinas como la benzodopa, la carboquona, la meturedopa y la uredopa; las etiloniminas y metilamelaminas incluyendo la altretamina, la trietilenomelamina, la trietilenofosforamida, la trietilenotiofosforamida y la trimetilolomelamina; los acetogeninos (en especial, el bullatacín y la bullatacinona); el delta-9-tetrahidrocannabinol (dronabinol, MARINOL®); la beta-lapachona; el lapachol; las colchicinas; el ácido betulínico, una camptotecina (incluyendo el topotecán análogo sintético (HYCAMTIN®), el CPT-11 (irinotecán, CAMPTOSAR®), la acetilcamptotecina, la escopolectina y la 9-aminocamptotecina); la

briostatina; la callistatina; el CC-1065 (incluyendo sus análogos sintéticos adocelesina, carcelesina y bicelesina); la podofilotoxina; el ácido podofilínico; la teniposida; las criptoficinas (particularmente la criptoficina 1 y la 8); la dolastatina; la duocarmicina (incluyendo los análogos sintéticos KW-2189 y CB1-TM1); la eleuterobina; la pancratistatina; una sarcodictina; la espongistatina; los gases nitrógeno como el clorambucil, la clornafacina, la colofosfamida, la estramustina, la ifosfamida, la mecloretamina, el clorhidrato de óxido de meclore'amina, el melfalán, la novembichina, la fenesterina, la prednimustina, la trofosfamida y la uramustina; las nitrosureas como la carmustina, la clorozotocina, la fotemustina, la lomustina, la nimustina y la ranimustina; los antibióticos como los de enedina (por ejemplo, la caliqueamicina, especialmente la caliqueamicina gamma 1I y la omega I1 (véase, por ejemplo, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); la dinemicina, incluyendo la dinemicina A; una esperamicina; el cromóforo de neocarcinostatina y los cromóforos de antibióticos de enedina cromoproteína relacionados (las aclacinomisinas, la actinomicina, la autramicina, la azaserina, las bleomicinas, la cactinomicina, la carabicina, la carminomicina, la carcinofilina, las cromomicinas, la dactinomicina, la daunorrubicina, la detorrubicina, la 6-diazo-5-oxo-L-norleucina, la doxorrubicina (incluyendo ADRIAMYCIN®, la morfolino-doxorrubicina, la cianomorfolino-doxorrubicina, la 2-pirrolino-doxorrubicina y la desoxidoxorrubicina), la epirrubicina, la esorrubicina, la idarrubicina, la marcelomicina, las mitomicinas como la mitomicina C, el ácido micofenólico, la nogalamicina, las olivomicinas, la peplomicina, la porfiromicina, la puromicina, la quelamicina, la rodorrubicina, la estreptonigrina, la estreptozocina, la tubercidina, el ubenimex, la cinostatina y la zorrubicina); los antimetabolitos como el metotrexato y el 5-fluorouracilo (5-FU); los análogos del ácido fólico como la denopterina, el metotrexato, la teropterina y el trimetrexato; los análogos de la purina como la fludarabina, la 6-mercaptopurina, la tiamiprina y la tioguanina; los análogos de la pirimidina como la ancitabina, la azacitidina, la 6-azauridina, el carmofur, la citarabina, la didesoxiuridina, la doxifluridina, la enocitabina y la floxuridina; los andrógenos como la calusterona, el propionato de dromostanolona, el epitiostanol, el mepitiostano, la testolactona los antiadrenérgicos como la aminoglutetimida, el mitotano y el trilostano; un reabastecedor del ácido fólico como el ácido folínico; la aceglatona; el glucósido aldofosfamida; el ácido aminolevulínico; el eniluracilo; la amsacrina; el bestrabucil; el bisantreno; el edatraxato; la defofamina; la demecolcina; la diaziquona; la elfornitina; el acetato de eliptinio; una epotilona; el etoglúcido; el nitrato de galio; la hidroxiurea; el lentinan; la lonidainina; los maitansinoides como la maitansina y las ansamitocinas; la mitoguazona; la mitoxantrona; el mopidanmol; la nitraerina; la pentostatina; el fenamet; la pirarrubicina; la losoxantrona; la 2-etilhidracida; la procarbacina; el complejo polisacárido PSK® (JHS Natural Products, Eugene, OR); el razoxano; la rizoxina; el sizofiran; el espirogermanio; el ácido tenuazónico; la triaciquona; la 2,2',2"-triclorotrietilamina; los tricotecenos (especialmente la toxina T-2, el verracurín A, el roridín A y la anguidina); el uretano; la vindesina (ELDISINE®, FILDESIN®); la dacarbacina; la manomustina; el mitobronitol; el mitolactol; el pipobromán; la gacitosina; la arabinosida ("Ara-C"); la tiotepa; los taxoides,

por ejemplo, el paclitaxel (TAXOL®) (Bristol-Myers Squibb Oncology, Princeton, N.J.), la formulación de nanopartículas de paclitaxel mediante ingeniería de albúmina libre de Cremofor (ABRAXANE™) (American Pharmaceutical Partners, Schaumberg, Illinois) y el docetaxel (TAXOTERE®) (Rhône-Poulenc Rorer, Antony, Francia); el clorambucil; la gemcitabina (GEMZAR®); la 6-tioguanina; la mercaptopurina; el metatrexato; los análogos de platino como el cisplatino y el carboplatino; la vinblastina (VELBAN®); el platino; el etopóido (VP-16); la ifosfamida; la mitoxantrona; la vincristina (ONCOVIN®); el oxaliplatino; la leucovovina; la vinorrelbina (NAVELBINE®); la novantrona; el edatrexato; la daunomicina; la aminopterina; el ibandronato; el inhibidor RFS 2000 de la topoisomerasa; la difluorometilornitina (DMFO); los retinoides como el ácido retinoico; capecitabina (XELODA®); las sales, los ácidos o los derivados farmacéuticamente aceptables de cualquiera de las sustancias anteriores, así como las combinaciones de una o más de las sustancias anteriores como la CHOP, la abreviatura de una terapia combinada de ciclofosfamida, doxorrubicina, vincristina y prednisolona, y la FOLFOX, la abreviatura de un régimen de tratamiento con oxaliplatino (ELOXATIN™) combinado con 5-FU y leucovovina.

[0216] También se incluyen en esta definición los agentes antihormonales que regulan, reducen, bloquean o inhiben los efectos de las hormonas que pueden fomentar el crecimiento del cáncer y se presentan a menudo en la forma de un tratamiento sistémico o que afecta a todo el cuerpo. Estos agentes podrán ser hormonas. Como ejemplos se incluyen los antiestrógenos y los moduladores selectivos de receptores de estrógenos (SERM), incluyendo, por ejemplo, el tamoxifeno (también el tamoxifeno NOLVADEX®), el raloxifeno (EVISTA®), el droloxifeno, el 4-hidroxitamoxifeno, el trioxifeno, el keoxifeno, el LY117018, la onapristona y el toremifeno (FARESTON®); las antiprogesteronas; los reguladores a la baja de los receptores de estrógenos (ERD); los agentes que oprimen o cierran los ovarios, por ejemplo, los agonistas de la hormona liberadora de la hormona luteinizante (LHRH) como el acetato de leuprolida (LUPRON® y ELIGARD®), el acetato de goserelina, el acetato de buserelina y la tripterelina; otros antiandrógenos como la flutamida, la nilutamida y la bicalutamida y los inhibidores de la aromatasa que inhiben la enzima aromatasa, encargada de regular la producción de estrógenos en las glándulas suprarrenales, como, por ejemplo, las 4(5)-imidazolas, la aminoglutetimida, el acetato de megestrol (MEGASE®), el exemestano (AROMASIN®), la formestania, la fadrozola, la vorozola (RIVISOR®), el letrozol (FEMARA®) y la anastrozola (ARIMIDEX®). Además, esta definición de los agentes quimioterapéuticos incluye los bisfosfonatos como el clodronato (por ejemplo, BONEFOS® o OSTAC®), el etidronato (DIDROCAL®), el NE-58095, el ácido zoledrónico/zoledronato (ZOMETA®), el alendronato (FOSAMAX®), el pamidronato (AREDIA®), el tiludronato (SKELID®) o el risedronato (ACTONEL®), así como la troxacitabina (un análogo de la citosina nucleásida 1,3-dioxolano); los oligonucleítidos antisentido, en particular los que inhiben la expresión génica en las rutas de señalización implicadas en la proliferación celular aberrante, como, por ejemplo, el PKC-alfa, el Raf, el H-Ras y el receptor del factor de

crecimiento epidérmico (EGF-R); las vacunas como THERATOPE® y las que se aplican para terapias génicas, por ejemplo, la ALLOVECTIN®, la LEUVECTIN® y la VAXID®; el inhibidor de la topoisomerasa 1 (por ejemplo, LURTOTECAN®); el rmRH (por ejemplo, ABARELIX®); el ditosilato de lapatinib (un inhibidor de molécula pequeña doble tirosincinasa del ErbB-2 y el EGFR conocido también como GW572016) y las sales, los ácidos o los derivados farmacéuticamente aceptables de cualquiera de las sustancias anteriores.

[0217] En la presente memoria descriptiva, "agente inhibidor del crecimiento" se refiere, a un compuesto o composición que inhibe el crecimiento de una célula (como una célula que exprese STEAP-1) tanto *in vitro* como *in vivo*. Así, el agente inhibidor del crecimiento puede reducir significativamente el porcentaje de células que expresen (como una célula que exprese STEAP-1) en la fase S. Ejemplos de agentes inhibidores del crecimiento serían los agentes que bloquean la progresión del ciclo celular (en un punto distinto de la fase S), como los agentes que inducen la detención de la G1 y la fase M. Entre los bloqueadores clásicos de la fase M se incluyen las vincas (vincristina y vinblastina), los taxanos y los inhibidores de la topoisomerasa II como la doxorrubicina, la epirrubicina, la daunorrubicina, el etopósido y la bleomicina. Esos agentes que detienen la G1 también se desbordan en la detención de la fase S, por ejemplo, los agentes de alquilación del ADN como el tamoxifeno, la prednisona, la dacarbacina, la mecloretamina, el cisplatino, el metotrexato, el 5-fluorouracilo y el ara-C. Puede encontrarse más información al respecto en *The Molecular Basis of Cancer*, Mendelsohn e Israel, eds., Capítulo 1, titulado "Cell cycle regulation, oncogenes, and antineoplastic drugs" por Murakami y col., (WB Saunders: Philadelphia, 1995), especialmente en la pág. 13. Los taxanos (paclitaxel y docetaxel) son antineoplásicos derivados ambos del tejo del Pacífico. El docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derivado del tejo europeo, es un análogo semisintético del paclitaxel (TAXOL®, Bristol-Myers Squibb). El paclitaxel y el docetaxel promueven la unión de microtúbulos a partir de dímeros de tubulina y estabilizan los microtúbulos al evitar la despolimerización, que ocasiona la inhibición de mitosis en células.

[0218] Por "metabolito intracelular" se entiende un compuesto que se deriva de un proceso o reacción metabólicos que se produce en el interior de una célula en un conjugado de anticuerpos con fármacos (ADC). El proceso o reacción metabólico puede ser un proceso enzimático, como escisión proteolítica de un enlazador peptídico del ADC, o hidrolisis de un grupo funcional, como una hidrazona, un éster o una amida. Los metabolitos intracelulares incluyen anticuerpos y fármacos libres que han sufrido escisión intracelular tras entrada, difusión, absorción o transporte a una célula.

[0219] Por "escindido intracelularmente" y "escisión intracelular" se entiende un proceso o reacción metabólicos en el interior de una célula de un conjugado de anticuerpos con fármacos (ADC) donde se rompe el enlace covalente, es decir, el enlazador, entre el fragmento del fármaco (D) y el

anticuerpo (Ab), lo que da lugar a que el fármaco libre se disocie del anticuerpo en el interior de la célula. Los fragmentos escindidos del ADC son, por lo tanto, metabolitos intracelulares.

[0220] Por "biodisponibilidad" se entiende la disponibilidad sistémica (es decir, concentración en sangre/plasma) de una cantidad determinada de fármaco administrada a un paciente. El término biodisponibilidad tiene un carácter absoluto e indica medición tanto del tiempo (velocidad) como de la cantidad total (alcance) del fármaco que alcanza la circulación general a partir de una dosis administrada.

[0221] Por "actividad citotóxica" se entenderá un efecto inhibidor del crecimiento, citostático o de destrucción celular de un conjugado de anticuerpos con fármacos o un metabolito intracelular de un conjugado de anticuerpos con fármacos. La actividad citotóxica se puede expresar como el valor de IC_{50} que es la concentración (molar o masa) por unidad de volumen a la que sobreviven la mitad de las células.

[0222] "Alquilo" es hidrocarbono C_1-C_{18} que contiene átomos de carbono normales, secundarios, terciarios o cíclicos. Los ejemplos incluyen metilo (Me, -CH3), etilo (Et, -CH2CH3), 1-propilo (n-Pr, n-propilo, -CH2CH2CH3), 2-propilo (i-Pr, i-propilo, -CH(CH3)2), 1-butilo (n-Bu, n-butilo, -CH2CH2CH2CH3), 2-metil-1-propilo (i-Bu, i-butilo, -CH2CH(CH3)2), 2-butilo (s-Bu, s-butilo, -CH(CH3)CH2CH3), 2-metil-2-propilo (t-Bu, t-butilo, -C(CH3)3), 1-pentil (n-pentil, -CH2CH2CH2CH2CH3), 2-pentil (-CH(CH3)CH2CH2CH3), 3-pentil (-CH(CH2CH3)2), 2-metil-2-butilo (-C(CH3)2CH2CH3), 3-metil-2-butilo (-CH(CH3)CH(CH3)2), 3-metil-1-butilo (-CH2CH2CH(CH3)2), 2-metil-1-butilo (-CH2CH(CH3)CH2CH3), 1-hexilo (-CH2CH2CH2CH2CH2CH3), 2-hexilo (-CH(CH3)CH2CH2CH2CH3), 3-hexilo (-CH(CH2CH3)(CH2CH2CH3)), 2-metil-2-pentilo (-C(CH3)2CH2CH2CH3), 3-metil-2-pentilo (-CH(CH3)CH(CH3)CH2CH3), 4-metil-2-pentilo (-CH(CH3)CH2CH(CH3)2), 3-metil-3-pentilo (-C(CH3)(CH2CH3)2), 2-metil-3-pentilo (-CH(CH2CH3)CH(CH3)2), 2,3-dimetil-2-butilo (-C(CH3)2CH(CH3)2), 3,3-dimetil-2-butilo (-CH(CH3)C(CH3)3.

[0223] En la presente memoria descriptiva, "C_1-C_8 alquilo" se refiere a un hidrocarbono de cadena recta o ramificada, saturado o no saturado, con 1 a 8 átomos de carbono. Entre los grupos "C_1-C_8 alquilo" representantivos se incluyen -metilo, -etilo, -n-propilo, -n-butilo, -n-pentilo, -n-hexilo, -n-heptilo, -n-octilo, -n-nonilo y -n-decilo; mientras que los grupos C_1-C_8 alquilo incluyen -isopropilo, -*sec*-butilo, -isobutilo, -*tert*-butilo, -isopentilo, 2-metilbutilo; los grupos no saturados C_1-C_8 alquilo incluyen -vinilo, -allilo, -1-butenilo, -2-butenilo, -isobutilenilo, -1-pentenilo, -2-pentenilo, -3-metilo-1-butenilo, -2-metilo-2-butenilo, -2,3-dimetil-2-butenilo, 1-hexilo, 2-hexilo, 3-hexilo,-acetilenilo, -propinilo, -1-butinilo, -2-butinilo, -1-pentinilo, -2-pentinilo, -3-metil-1 butinilo, metilo, etilo, propilo, isopropilo, *n*-butilo, isobutilo, *sec*-butilo, *tert*-butilo, *n*-pentilo, isopentilo, neopentilo, *n*-hexilo, isohexilo, 2-metilpentilo, 3-metilpentilo, 2,2-dimetilbutilo, 2,3-dimetilbutilo, 2,2-dimetilpentilo, 2,3-dimetilpentilo, 3,3-dimetilpentilo, 2,3,4-trimetilpentilo, 3-metilhexilo, 2,2-dimetilhexilo, 2,4-dimetilhexilo, 2,5-dimetilhexilo,

3,5-dimetilhexilo, 2,4-dimetilpentilo, 2-metilheptilo, 3-metilheptilo, *n*-heptilo, isoheptilo, *n*-octilo, e isooctilo. Un grupo C_1-C_8 alquilo se puede sustituir o no con uno o más grupos, incluidos, -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R', -SO_3R', -$S(O)_2$R', -S(O)R', -OH, -halógeno, -N_3 , -NH_2, -NH(R'), -$N(R')_2$ y -CN; donde cada R' se selecciona independientemente entre H, -C_1-C_8 alquilo y arilo.

[0224] "Alquenil" es hidrocarbono C_2-C_{18} que contiene átomos de carbono normales, secundarios, terciarios o cíclicos con al menos un sitio de insaturación, es decir, un enlace doble sp^2 carbono-carbono. Entre los ejemplos, están: etileno o vinilo (-CH=CH_2), alil (-CH_2CH=CH_2), ciclopentenil (-C_5H_7) y 5-hexenil (-CH_2 $CH_2CH_2CH_2$CH=CH_2)

[0225] "Alquinil" es hidrocarbono C_2-C_{18} que contiene átomos de carbono normales, secundarios, terciarios o cíclicos con al menos un sitio de insaturación, es decir, un enlace triple sp carbono-carbono. Entre los ejemplos, están: acetilénico (-C≡CH) y propargil (-CH_2C≡CH),

[0226] Por "alquileno" se entiende un radical hidrocarbono saturado, cíclico o de cadena recta o ramificada de 1 a 18 átomos de carbono, con dos centros radicales monovalentes derivados mediante la eliminación de dos átomos de hidrógeno del mismo átomo o de dos átomos de carbono distintos de un alcano padre. Entre los radicales alquilenos típicos, están: metileno (-CH_2-) 1,2-etil (-CH_2CH_2-), 1,3-propil (-$CH_2CH_2CH_2$-), 1,4-butilo (-$CH_2CH_2CH_2CH_2$-), etc.

[0227] Un "C_1-C_{10} alquileno" es un grupo hidrocarbono saturado de cadena recta de la fórmula -$(CH_2)_{1-10}$-. Ejemplos de un C_1-C_{10} alquileno son metileno, etileno, propileno, butileno, pentileno, hexileno, heptileno, ocitileno, nonileno y decaleno.

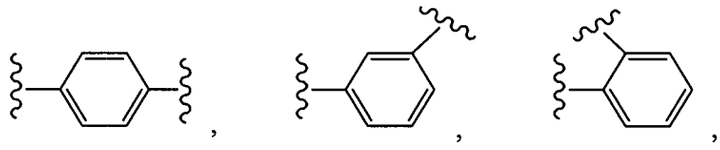
[0228] Por "alquenileno" se entiende un radical hidrocarbono insaturado, cíclico o de cadena recta o ramificada de 2 a 18 átomos de carbono, con dos centros radicales monovalentes derivados mediante la eliminación de dos átomos de hidrógeno del mismo átomo o de dos átomos de carbono distintos de un alqueno padre. Entre los radicales alquenilenos típicos, están: 1,2-etileno (-CH=CH-).

[0229] Por "alquinileno" se entiende un radical hidrocarbono insaturado, cíclico o de cadena recta o ramificada de 2 a 18 átomos de carbono, con dos centros radicales monovalentes derivados mediante la eliminación de dos átomos de hidrógeno del mismo átomo o de dos átomos de carbono distintos de un alquino padre. Entre los radicales alquinilenos típicos, están: acetileno (-C≡C-), propargil (-CH_2C≡C-) y 4-pentinil (-$CH_2CH_2CH_2$C≡C-).

[0230] Por "arilo" se entiende un grupo aromático carbocíclico. Ejemplos de grupos arilo se incluyen, entre otros, fenilo, naftilo y antracenilo, entre otros. Un grupo aromático carbocíclico o un grupo aromático heterocíclico se puede sustituir o no con uno o más grupos, incluidos, -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', -$C(O)NH_2$, -C(O)NHR', -$C(O)N(R')_2$ -NHC(O)R',

$-SO_3R'$, $-S(O)_2R'$, -S(O)R', -OH, -halógeno, $-N_3$, $-NH_2$, -NH(R'), $-N(R')_2$ y -CN; donde cada R' se selecciona independientemente entre H, $-C_1-C_8$ alquilo y arilo.

[0231] Un "arileno" es un grupo arilo que tiene dos enlaces covalentes y puede estar en configuraciones orto, meta o para, como se muestra en las estructuras siguientes:



en el que el grupo fenilo se puede sustituir o no con hasta cuatro grupos, incluidos, $-C_1-C_8$ alquilo, $-O-(C_1-C_8$ alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', $-C(O)NH_2$, -C(O)NHR', $-C(O)N(R')_2$ -NHC(O)R', $-SO_3R'$, $-S(O)_2R'$, -S(O)R', -OH, -halógeno, $-N_3$, $-NH_2$, -NH(R'), $-N(R')_2$ y -CN; donde cada R' se selecciona independientemente entre H, $-C_1-C_8$ alquilo y arilo.

[0232] Por "arilalquilo" se entiende un radical alquilo acíclico en el que uno de los átomos de hidrógeno unido a un átomo de carbono, por lo general un átomo de carbono sp^3 o terminal, se sustituye por un radical arilo. Habitualmente, los grupos arilalquil incluyen benzil, 2-feniletan-1-il, 2-fenileten-1-il, naftilmetil, 2-naftiletan-1-il, 2-naftileten-1-il, naftobenzil, 2-naftofeniletan-1-il y similares. El grupo arilalquil comprende de 6 a 20 átomos de carbono, por ejemplo, la fracción alquilo, incluidos los grupos alcanil, alquenil o alquinil, del grupo arilalquil está formada por 1 a 6 átomos de carbono y la porción arilo tiene de 5 a 14 átomos de carbono.

[0233] Por "heteroarilalquil" se entiende un radical alquilo acíclico en el que uno de los átomos de hidrógeno unido a un átomo de carbono, por lo general un átomo de carbono sp^3 o terminal, se sustituye por un radical heteroaril. Los grupos heteroarilalquil típicos incluyen 2-benzimidazolilmetil, 2-furiletil y similares, entre otros. El grupo heteroarilalquil comprende de 6 a 20 átomos de carbono, por ejemplo, la fracción alquilo, incluidos los grupos alcanil, alquenil o alquinil, del grupo heteroarilalquil contiene de 1 a 6 átomos de carbono y la fracción heteroaril tiene de 5 a 14 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S. La fracción heteroaril del grupo hetroarilalquil puede ser un monociclo que tenga de 3 a 7 anillos (2 a 6 átomos de carbono o un biciclo con 7 a 10 anillos (4 a 9 átomos de carbono y 1 a 3 heteroátomos seleccionados entre N, O, P y S), por ejemplo: un sistema biciclo [4,5], [5,5], [5,6] o [6,6].

[0234] Por "alquilo sustituido", "arilo sustituido" y "arilalquilo osustituido" se entienden alquilo, arilo y arilalquilo respectivamente, en los que uno o más átomos de hidrógeno son sustituidos independientemente por un sustituto. Entre los sustitutos típicos están, -X, -R, $-O^-$, -OR, -SR, $-S^-$, $-NR_2$, $-NR_3$, =NR, $-CX_3$, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, $-NO_2$, $=N_2$, $-N_3$, NC(=O)R, -C(=O)R, $-C(=O)NR_2$,

$-SO_3^-$, $-SO_3H$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, $-S(=O)R$, $-OP(=O)(OR)_2$, $-P(=O)(OR)_2$, $-PO^-_3$, $-PO_3H_2$, $-C(=O)R$, $-C(=O)X$, $-C(=S)R$, $-CO_2R$, $-CO^{2-}$, $-C(=S)OR$, $-C(=O)SR$, $-C(=S)SR$, $-C(=O)NR_2$, $-C(=S)NR_2$, $-C(=NR)NR_2$, entre otros, donde cada X es independientemente un halógeno: F, Cl, Br o I; y cada R es independientemente -H, C_2-C_{18} alquilo, C_6-C_{20} arilo, C_3-C_{14} heterociclo, grupo protector o fracción de profármaco. Los grupos alquileno, alquenileno y alquinileno descritos anteriormente también se pueden sustituir de forma similar.

[0235] Por "heteroaril" y "heterociclo" se entiende un sistema de anillos en el que uno o más átomos en anillo es un heteroátomo, por ejemplo, nitrógeno, oxígeno y sulfuro. El radical heterociclo comprende de 1 a 20 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S. Un heterociclo puede ser un monociclo con 3 a 7 anillos (de 2 a 6 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S) o un biciclo con 7 a 10 anillos (de 4 a 9 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S), por ejemplo: un sistema biciclo [4,5], [5,5], [5,6] o [6,6].

[0236] Los heterociclos se describen en Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* (W.A. Benjamin, New York, 1968), especialmente en los Capítulos 1, 3, 4, 6, 7 y 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley and Sons, New York, 1950 hasta hoy), en concreto en los Volúmenes 13, 14, 16, 19 y 28; y *J. Am. Chem. Soc.* (1960) 82:5566.

[0237] Ejemplos de heterociclos son piridil, dihidroipiridil, tetrahidropiridil (piperidil), tiazolil, tetrahidrotiofeno, tetrahidrotiofeno de azufre oxidado, pirimidinil, furanil, tienil, pirrolil, pirazolil, imidazolil, tetrazolil, benzofuranil, tianaftalenil, indolil, indolenil, quinolinil, isoquinolinil, benzimidazolil, piperidinil, 4-piperidonil, pirrolidinil, 2-pirrolidonil, pirrolinil, tetrahidrofuranil, bis-tetrahidrofuranil, tetrahidropiranil, bis-tetrahidropiranil, tetrahidroquinolinil, tetrahidroisoquinolinil, decahidroquinolinil, octahidroisoquinolinil, azocinil, triazinil, 6H-1,2,5-tiadiazinil, 2H,6H-1,5,2-ditiazinil, tienil, tiantrenil, piranil, isobenzofuranil, chromenil, xantenil, fenoxatinil, 2H-pirrolil, isotiazolil, isoxazolil, pirazinil, piridazinil, indolizinil, isoindolil, 3H-indolil, 1H-indazolil, purinil, 4H-quinolizinil, ftalazinil, naftiridinil, quinoxalinil, quinazolinil, cinnolinil, pteridinil, 4aH-carbazolil, carbazolil, β-carbolinil, fenantridinil, acridinil, pirimidinil, fenantrolinil, fenazinil, fenotiazinil, furazanil, fenoxazinil, isochromanil, chromanil, imidazolidinil, imidazolinil, pirazolidinil, pirazolinil, piperazinil, indolinil, isoindolinil, quinuclidinil, morfolinil, oxazolidinil, benzotriazolil, benzisoxazolil, oxindolil, benzoxazolinil e isatinoil.

[0238] A modo de ejemplo, los heterociclos con enlace a carbono se enlazan en la posición 2, 3, 4, 5 ó 6 de una piridina, posición 3, 4, 5 ó 6 de una piridazina, posición 2, 4, 5 ó 6 de una pirimidina, posición 2, 3, 5 ó 6 de una pirazina, posición 2, 3, 4 ó 5 de un furano, tetrahidrofurano, tiofurano, tiofeno, pirrol o tetrahidropirrol, posición 2, 4 ó 5 de una oxazola, imidazola o tiazola, posición 3, 4 ó 5 de una isoxazola, pirazola o isotiazola, posición 2 ó 3 de una aziridina, posición 2, 3 ó 4 de una azetidina, posición 2, 3, 4, 5, 6, 7 u 8 de una quinolina o posición 1, 3, 4, 5, 6, 7 u 8 de una isoquinolina. Aún es más habitual que los

heterociclos con enlace a carbono incluyan 2-piridil, 3-piridil, 4-piridil, 5-piridil, 6-piridil, 3-piridazinil, 4-piridazinil, 5-piridazinil, 6-piridazinil, 2-pirimidinil, 4-pirimidinil, 5-pirimidinil, 6-pirimidinil, 2-pirazinil, 3-pirazinil, 5-pirazinil, 6-pirazinil, 2-tiazolil, 4-tiazolil o 5-tiazolil.

[0239] A modo de ejemplo, los heterociclos con enlace a nitrógenos están enlazados en la posición 1 de una aziridina, azetidina, pirrol, pirrolidina, 2-pirrolina, 3-pirrolina, imidazol, imidazolidina, 2-imidazolina, 3-imidazolina, pirazol, pirazolina, 2-pirazolina, 3-pirazolina, piperidina, piperazina, indol, indolina, 1H-indazol, posición 2 de un isoindol o isoindolina, posición 4 de una morfolina, y posición 9 de un carbazol o β-carbolina, entre otros. Aún es más típico que los heterocíclos con enlace a nitrógeno incluyan 1-aziridil, 1-azetedil, 1-pirrolil, 1-imidazolil, 1-pirazolil, y 1-piperidinil.

[0240] Por "C_3-C_8 heterociclo" se entiende un C_3-C_8 carbociclo aromático o no aromático en el que de uno a cuatro de los átomos de carbono en anillo son sustituidos independientemente por un heteroátomo del grupo formado por O, S y N. Ejemplos representativos de un C_3-C_8 heterociclo incluyen benzofuranil, benzotiofeno, indolil, benzopirazolil, coumarinil, isoquinolinil, pirrolil, tiofenil, furanil, tiazolil, imidazolil, pirazolil, triazolil, quinolinil, pirimidinil, piridinil, piridonil, pirazinil, piridazinil, isotiazolil, isoxazolil y tetrazolil. Un C_3-C_8 heterociclo se puede sustituir o no con hasta siete grupos, incluidos, -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -halógeno, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ y -CN; donde cada R' se selecciona independientemente entre H, -C_1-C_8 alquilo y arilo.

[0241] Por "C_3-C_8 heterociclo" se entiende un grupo C_3-C_8 heterociclo como se define anteriormente, donde uno de los átomos de hidrógeno del grupo heterociclo es sustituido por un enlace. Un C_3-C_8 heterociclo se puede sustituir o no con hasta seis grupos, incluidos, -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -halógeno, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ y -CN; donde cada R' se selecciona independientemente entre H, -C_1-C_8 alquilo y arilo.

[0242] Por "carbociclo" se entiende un anillo saturado o insaturado con 3 a 7 átomos de carbono como monociclo o 7 a 12 átomos de carbono como biciclo. Los carbociclos monociclicos tienen de 3 a 6 átomos en anillo y aún con más frecuencia 5 ó 6 átomos en anillo. Los carbociclos bicíclicos tienen de 7 a 12 átomos en anillo, por ejemplo, dispuestos como un sistema biciclo [4,5], [5,5], [5,6] o [6,6] o de 9 a 10 átomos en anillo dispuestos como un sistema biciclo [5,6] o [6,6]. Ejemplos de carbociclos monocíclicos incluyen ciclopropil, ciclobutil, ciclopentil, 1-ciclopent-1-enil, 1-ciclopent-2-enil, 1-ciclopent-3-enil, ciclohexil, 1-ciclohex-1-enil, 1-ciclohex-2-enil, 1-ciclohex-3-enil, cicloheptil y ciclooctil.

[0243] Un "C_3-C_8 carbociclo" es un anillo carbocíclico no aromático, saturado o insaturado, de 3-, 4-, 5-, 6-, 7- u 8- átomos. Entre los C_3-C_8 carbociclos representativos están -ciclopropil, -ciclobutil, -ciclopentil,

-ciclopentadienil, -ciclohexil, -ciclohexenil, -1,3-ciclohexadienil, -1,4-ciclohexadienil, -cicloheptil, -1,3-cicloheptadienil, -1,3,5-cicloheptatrienil, -ciclooctil y -ciclooctadienil. Un grupo C_3-C_8 carbociclo se puede sustituir o no con uno o más grupos, incluidos, -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -arilo, -C(O)R', -OC(O)R', -C(O)OR', -C(O)NH_2 , -C(O)NHR', -C(O)N(R')$_2$ -NHC(O)R', -SO_3R', -S(O)$_2$R', -S(O)R', -OH, -halógeno, -N_3 , -NH_2, -NH(R'), -N(R')$_2$ y -CN; donde cada R' se selecciona independientemente entre H, -C_1-C_8 alquilo y arilo.

[0244] Por "C_3-C_8 carbociclo" se entiende un grupo C_3-C_8 carbociclo como se define anteriormente, donde uno de los átomos de hidrógeno del grupo carbociclo es sustituido por un enlace.

[0245] Por "enlazador" se entiende una fracción química que comprende un enlace covalente o una cadena de átomos que unen covalentemente un anticuerpo a una fracción de un fármaco. En varias formas de realización, los enlazadores incluyen un radical divalente, como un alquildiil, un arildiil, un heteroarildiil, fracciones como: $-(CR_2)_nO(CR_2)_n-$, unidades repetidoras de alquiloxi (por ejemplo, polietilenoxi, PEG, polimetileneoxi) y alquilamino (por ejemplo, polietileneamino, Jeffamine™); y éster diácido y amidas como succinato, succinamida, diglicolato, malonato y caproamida.

[0246] Por "quiral" se entiende moléculas que tienen la propiedad de no superimposición de la molécula de enlace que constituye la contraimagen, mientras que por "aquiral" se entiende las moléculas que son superponibles a su contraimagen.

[0247] Por "estereoisomeros" se entienden los compuestos que tienen idéntica constitución química pero difieren en la disposición de los átomos o grupos en el espacio.

[0248] Por "diastereomero" se entiende un estereosiomero con dos o más centros de quiralidad y con moléculas que no son contraimágenes la una de la otra. Los diastereomeros tienen propiedades físicas distintas, por ejemplo, puntos de fusión, puntos de ebullición, propiedades espectrales y reactividades. Las mezclas de diastereomeros se pueden separar mediante procedimientos analíticos de alta resolución, como electroforesis y cromatografía.

[0249] Por "enantiomeros" se entiende dos estereoisomeros de un compuesto que no se superponen como contraimagen el uno del otro.

[0250] Las definiciones y convenciones estereoquímicas usadas en la presente memoria descriptiva siguen, por lo general, a S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; y Eliel, E. y Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Existen muchos compuestos orgánicos en formas ópticamente activas, es decir, con capacidad para rotar el plano a luz polarizada en el plano. Al describir un compuesto ópticamente activo, se utilizan los prefijos D y L, o R y S, para denotar la configuración absoluta de la molécula sobre su centro o sus centros quirales. Los prefijos d y l o (+) y (-) se emplean para designar el

signo de rotación de luz polarizada/plano por el compuesto, donde (-) o l significan que el compuesto es levorotarorio. Un compuesto con prefijo (+) o d es dextrorotatorio. Para una estructura química dada, estos estereoisomeros son idénticos, excepto que sean contraimagen el uno del otro. Un esteroisomero específico también se puede considerar un enantiomero y una mezcla de dichos isomeros se denomina con frecuencia una mezcla enantiomérica. Una mezcla de enantiomeros en una proporción 50:50 se considera una mezcla racémica o un racemato, que puede ocurrir cuando no ha habido estereoselección o estereoespecificidad en una reacción o proceso químico. Por "mezcla racémica" o "racemato" se entiende una mezcla equimolar de dos especies enatioméricas, desprovista de actividad óptica.

[0251] Por "grupo saliente" se entiende un grupo funcional que se puede sustituir por otro grupo funcional. Determinados grupos salientes son bien conocidos en el sector y entre los ejemplos están un halido (por ejemplo, cloruro, bromuro, yoduro), metanesulfonil (mesil), p-toluenesulfonil (tosil), trifluorometilsulfonil (triflato) y trifluorometilsulfonato.

Abreviaturas

[0252] COMPONENTES DEL ENLAZADOR:

MC = 6-maleimidocaproil

Val-Cit o "vc" = valina-citrulina (un dipéptido ejemplar en un enlazador escindible por una proteasa)

Citrulina = ácido 2-amino-5-ureido pentanóico

PAB = p-aminobenziloxicarbonilo (un ejemplo de un componente enlazador "autoinmolador")

Me-Val-Cit = N-metil-valina-citrulina (donde el enlace peptídico del enlazador se ha modificado para prevenir su escisión mediante catepsina B)

MC(PEG)6-OH = maleimidocaproil- polietilenglicol (se puede unir a cisteínas del anticuerpo).

SPP = N-succinimidil-4-(2-piridiltio)pentanoato

SPDP = N-succinimidil-3-(2-piridilditio) propionato

SMCC = succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato

IT = iminotiolano

FÁRMACOS CITOTÓXICOS:

[0253] MMAE = mono-metil auristatina E (MW 718)

MMAF = variante de la auristatina E (MMAE) con una fenilalanina en el extremo C-terminal del fármaco (MW 731.5)

MMAF-DMAEA = MMAF con DMAEA (dimetilaminoetilamina) en un enlace de amida a la fenilalanina en el extremo C-terminal (MW 801.5)

MMAF-TEG = MMAF con tetraetilenglicol esterificado a la fenilalanina

MMAF-NtBu = N-t-butilo, unido como una amida al extremo C-terminal de MMAF

DM1 = N(2')-deacetil-N(2')-(3-mercapto-1-oxopropil)-maitansina

DM3 = N(2')-deacetil-N2-(4-mercapto-1-oxopentil)-maitansina

DM4 = N(2')-deacetil-N2-(4-mercapto-4-metil-1-oxopentil)-maitansina

[0254] Otras abreviaturas son: AE es auristatina E, Boc es *N*-(*t*-butoxicarbonil), cit es citrullina, dap is dolaproina, DCC es 1,3-diciclohexilcarbodiimida, DCM es diclorometano, DEA es dietilamina, DEAD es dietilazodicarboxilato, DEPC es dietilfosforilcianidato, DIAD es diisopropilazodicarboxilato, DIEA es *N,N*-diisopropiletilamina, dil es dolaisoleucina, DMA es dimetilacetamida, DMAP es 4-dimetilaminopiridina, DME es etileneglicol dimetil éter (o 1,2-dimetoxietano), DMF es *N,N*-dimetilformamida, DMSO es dimetilsulfoxido, doe es dolafenina, dov es *N,N*-dimetilvalina, DTNB es 5,5'-ditiobis(2-ácido nitrobenzoico), DTPA es ácido dietilenetriaminepentaacético, DTT es ditiotreitol, EDCI es hidrocloruro de 1-(3-dimetilaminopropil)-3-etilcarbodiimida, EEDQ es 2-etoxi-1-etoxicarbonil-1,2-dihidroquinolina, ES-MS es espectrometría de masas por electrospray, EtOAc es acetato de etilo, Fmoc es *N*-(9-fluorenilmetoxicarbonil), gly es glicina, HATU es *O*-(7-azabenzotriazol-1-il)-*N,N,N',N'*-tetrametiluronium hexafluorofosfato, HOBt es 1-hidroxibenzotriazol, HPLC es cromatografía líquida de alta presión, ile es isoleucina, lys es lisina, MeCN (CH_3CN) es acetonitrilo, MeOH es metanol, Mtr es 4-anisildifenilmetil (o 4-metoxitritil), nor es (*1S, 2R*)-(+)-norefedrina, PBS es solución salina fosfatada (pH 7,4), PEG es polietilenglicol, Ph es fenilo, Pnp es p-nitrofenilo, MC es 6-maleimidocaproil, phe es L-fenilalanina, PyBrop es bromo *tris*-pirrolidino fosfonium hexafluorofosfato, SEC es cromatografía por exclusión de tamaño, Su es succinimida, TFA es ácido trifluoroacético, TLC es cromatografía de capa fina, UV es ultravioleta y val es valina.

COMPOSICIONES Y MÉTODOS DE FABRICACIÓN DE LAS MISMAS

[0255] Se proporcionan anticuerpos que se unen a STEAP-1. Se proporcionan inmunoconjugados que comprenden anticuerpos anti-STEAP-1. Los anticuerpos e inmunoconjugados de la invención son útiles, como por ejemplo, para el diagnóstico o tratamiento de trastornos asociados a una expresión alterada, como por ejemplo, una expresión aumentada, de STEAP-1. En determinadas formas de realización, los anticuerpos o inmunoconjugados de la invención son útiles para el diagnóstico o tratamiento de un trastorno de proliferación celular, como un cáncer.

Anticuerpos anti-STEAP-1

[0256] En un aspecto, la invención proporciona anticuerpos que se unen a STEAP-1. En algunas formas de realización, se proporcionan anticuerpos que se unen a una forma madura del STEAP-1 de humanos y monos cinomolgus. En una de estas formas de realización, una forma madura del STEAP-1 humano tiene una secuencia de aminoácidos de identificador de secuencia n.º: 1 (Figura 1). La STEAP-1 de mono tiene una secuencia de aminoácidos de identificador de secuencia n.º: 3 (Figura 1). En algunas formas de realización, un anticuerpo de STEAP-1 se une a una forma madura de STEAP-1 expresado en una superficie celular. En algunas formas de realización, un anticuerpo que se une a una forma madura de

STEAP-1 expresada en la superficie celular inhibe el crecimiento de la célula. En algunas formas de realización, un anticuerpo anti-STEAP-1 se une a una forma madura de STEAP-1 expresada en la superficie celular e inhibe la proliferación celular. En algunas formas de realización, un anticuerpo anti-STEAP-1 se une a una forma madura de STEAP-1 expresada en la superficie celular e induce la muerte celular. En algunas formas de realización, un anticuerpo anti-STEAP-1 se une a una forma madura de STEAP-1 expresada en la superficie de células cancerosas. En algunas formas de realización, un anticuerpo anti-STEAP-1 se una a una forma madura de STEAP-1 que está sobreexpresada en la superficie de células cancerosas con respecto a células normales del mismo origen tisular. En algunas formas de realización, el anticuerpo anti-STEAP-1 se conjuga a una citotoxina o a una marca detectable y se une al STEAP-1 en una superficie celular. En algunas formas de realización, el conjugado de anticuerpo con toxina inhibe el crecimiento de la célula. En algunas formas de realización, el conjugado de anticuerpo con una marca detectable produce una célula que expresa STEAP-1 en su superficie para que sea detectable *in vitro* o *in vivo*.

[0257] En un aspecto, un anticuerpo anti-STEAP-1 es un anticuerpo monoclonal. En un aspecto, un anticuerpo anti-STEAP-1 es un fragmento de anticuerpo, por ejemplo, un fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$. En un aspecto, un anticuerpo anti-STEAP-1 es un anticuerpo quimérico, humanizado o humano. En un aspecto, cualquiera de los anticuerpos anti-STEAP-1 descritos en la presente memoria descriptiva está purificado.

[0258] En la presente memoria descriptiva se proporcionan anticuerpos monoclonales ejemplares derivados de una biblioteca de fagos. El antígeno que se utilizó para la detección de la biblioteca era un polipéptido con la secuencia de aminoácidos de identificador de secuencia n.º: 28 o identificador de secuencia n.º: 30, que correspondía a los dominios extracelulares (ECD) del STEAP-1 beta y alfa. Los anticuerpos que se obtienen de la detección de la biblioteca tienen una maduración de afinidad.

[0259] En un aspecto, se proporcionan anticuerpos monoclonales que compiten con el anticuerpo murino 120.545,, injerto 120 y el humanizado 120v.24 para que se unan al STEAP-1. También se proporcionan anticuerpos monoclonales que se unen al mismo epítopo como el anticuerpo murino 120.545, 120, injerto 120 yel humanizado 120v.24.

[0260] En un aspecto de la invención, se proporcionan polinucleótidos que codifican anticuerpos anti-STEAP-1. En ciertas formas de realización, se proporcionan vectores que comprenden polinucleótidos que codifican anticuerpos anti-STEAP-1. En ciertas formas de realización, se proporcionan células hospederas que comprenden estos vectores. En otro aspecto de la invención, se proporcionan composiciones que comprenden anticuerpos anti-STEAP-1 o polinucleótidos que codifican anticuerpos anti-STEAP-1. En determinadas formas de realización, una composición de la invención es una

formulación farmacéutica para el tratamiento de un trastorno de proliferación celular, como los enumerados en la presente memoria descriptiva.

A continuación se incluye una descripción detallada de anticuerpos anti-STEAP-1 ejemplares:

1. Formas de realización específicas de anticuerpos anti-STEAP-1

[0261] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena pesada que comprende un identificador de secuencia n.º: 9 ó 10 de la Figura 2B. En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena ligera que comprende un identificador de secuencia n.º: 6 de la Figura 2A.

[0262] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende una cadena pesada que comprende un identificador de secuencia n.º: 9 que tiene uno o más de los siguientes cambios de aminoácidos en la posición de Kabat indicada: A24V, V37I, V48M, F67I y L78F. En una realización, la cadena pesada comprende una región de estructura de cadena pesada de los identificadores de secuencia Nºs. 25, 75, 76, 77, 78 y 79. Tal como se emplean en la presente memoria, las regiones de la estructura de la cadena pesada se designan "FR-H1-H4" o "HC-FR1-FR4", y las regiones de la estructura de la cadena ligera se designan "FR-L1-L4" o "LC-FR1-FR4". En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende una cadena ligera que comprende un identificador de secuencia n.º: 6.

[0263] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende 1, 2, 3, 4, 5 ó 6 de las secuencias de la HVR del anticuerpo 120.v24 como se muestra en las Figuras 2A y 2B.

[0264] Un anticuerpo anti-STEAP-1 puede comprender cualquier secuencia de dominio variable de la estructura adecuada, siempre que el anticuerpo conserve la capacidad de unirse a STEAP-1. Por ejemplo, en ciertas formas de realización, los anticuerpos anti-STEAP-1 de la invención comprenden una secuencia de consenso de la estructura de la cadena pesada del subgrupo III humano. En una forma de realización de estos anticuerpos, la secuencia de consenso de la estructura de la cadena pesada involucra sustituciones en la posición 24, 37, 48, 67, ó 78. En una forma de realización de estos anticuerpos, la posición 24 corresponde a A o V, la posición 37 corresponde a I o V, la posición 48 corresponde a M o V, la posisición 67 corresponde a I o F, y/o la posición 78 corresponde a F o L. En una forma de realización, estos anticuerpos comprenden una secuencia de estructura de dominio variable de la cadena pesada de huMAb4D5-8, por ejemplo, el identificador de secuencia n.º: 21, 22, 23, y 24 (FR-H1, FR-H2, FR-H3, FR-H4, respectivamente). huMAb4D5-8 se conoce comercialmente como el anticuerpo anti-HER2 HERCEPTIN®, Genentech, Inc., South San Francisco, CA, Estados Unidos; también mencionado en las patentes de EE.UU N.º 6,407,213 y 5,821,337; y en Lee y col., *J. Mol. Biol.* (2004), 340(5):1073-93. En dicha forma de realización, estos anticuerpos además comprenden una secuencia de consenso de la

estructura de cadena ligera κI humana. En una de esas formas de realización, estos anticuerpos comprenden una secuencia de la estructura de dominio variable de la cadena ligera de huMAb4D5-8, por ejemplo, el identificador de secuencia n.º: 17, 18, 19, y 20 (FR-L1, FR-L2, FR-L3, FR-L4, respectivamente).

[0265] En una forma de realización, un anticuerpo anti-STEAP-1 comprende un dominio variable de cadena pesada que comprende una secuencia de estructura y regiones hipervariables, en las que la secuencia de estructura comprende las secuencias FR-H1-FR-H4 del identificador de secuencia n.º: 21 ó 25 (FR-H1), 22 (FR-H2), 23 (FR-H3) y 24 (FR-H4) respectivamente; la HVR-H1 comprende la secuencia de aminoácidos del identificador de secuencia n.º: 14; la HVR-H2 comprende la secuencia de aminoácidos del identificador de secuencia n.º: 15 y la HVR-H3 comprende una secuencia de aminoácidos del identificador de secuencia n.º: 16. En una forma de realización, un anticuerpo anti-STEAP-1 comprende un dominio variable de cadena ligera que comprende una secuencia de estructura y regiones hipervariables, en las que la secuencia de estructura comprende las secuencias FR-L1-FR-L4 de los identificadores de frecuencia nº.: 17, 18, 19 y 20 respectivamente; la HVR-L1 comprende la secuencia de aminoácidos seleccionada del identificador de secuencia nº.: 11, 12 y 13. En una forma de realización de estos anticuerpos, el dominio variable de cadena pesada comprende un identificador de secuencia n.º: 9 ó 10 y el dominio variable de cadena ligera comprende el identificador de secuencia n.º: 6.

[0266] En ciertas formas de realización, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena pesada que comprende una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia con respecto a una secuencia de aminoácidos del identificador de secuencia n.º: 9 ó 10. En ciertas formas de realización, una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia contiene sustituciones, inserciones o eliminaciones relativas a la secuencia de referencia; sin embargo, un anticuerpo que comprende una secuencia de aminoácidos conserva la capacidad de unirse a STEAP-1. En ciertas formas de realización, un total de entre 1 y 10 aminoácidos se han sustituido, insertado o eliminado en una secuencia del identificador de secuencia n.º: 9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, y/o 79. En ciertas formas de realización, las sustituciones, inserciones o eliminaciones se producen en las regiones externas a las HVR (es decir, en las FR). En ciertas formas de realización, un anticuerpo anti-STEAP-1 comprende un dominio variable de cadena pesada que contiene una secuencia de aminoácidos del identificador de secuencia n.º: 9 ó 10.

[0267] En ciertas formas de realización, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena pesada, tal como se describe en la Figura 2B (Identificador de secuencia nº 9 ó 10).

[0268] En ciertas formas de realización, las secuencias de la HVR y FR de cadena pesada comprenden los siguientes elementos:

HVR-H1 (GYSITSDYAWN, identificador de secuencia n.º: 14)

HVR-H2 (GYISNSGSTSYNPSLKS, identificador de secuencia n.º: 15)

HVR-H3 (ERNYDYDDYYYAMDY, identificador de secuencia n.º: 16)

FR-H1 (EVQLVESGGGLVQPGGSLRLSCAAS, identificador de secuencia n.º: 21)

FR-H1 (EVQLVESGGGLVQPGGSLRLSCAVS, identificador de secuencia n.º: 25)

FR-H2 (WVRQAPGKGLEWV, identificador de secuencia n.º: 22)

FR-H2 (WIRQAPGKGLEWV, identificador de secuencia n.º: 75)

FR-H2 (WVRQAPGKGLEWM, identificador de secuencia n.º: 76)

FR-H2 (WIRQAPGKGLEWM, identificador de secuencia n.º: 77)

FR-H3 (RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR, identificador de secuencia n.º: 23)

FR-H3 (RITISRDNSKNTLYLQMNSLRAEDTAVYYCAR, identificador de secuencia n.º: 78)

FR-H3 (RFTISRDNSKNTFYLQMNSLRAEDTAVYYCAR, identificador de secuencia n.º: 79)

FR-H4 (WGQGTLVTVSS, identificador de secuencia n.º: 24)

[0269] En ciertas formas de realización, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena ligera, tal como se describe en la Figura 2A (Identificador de secuencia nº 6).

[0270] En ciertas formas de realización, las secuencias de la HVR de cadena ligera comprenden los siguientes elementos:

HVR-L1 (KSSQSLLYRSNQKNYLA, identificador de secuencia n.º: 11)

HVR-L2 (WASTRES, identificador de secuencia n.º:12)

HVR-L3 (QQYYNYPRT, identificador de secuencia n.º: 13).

[0271] En ciertas formas de realización, las secuencias de la FR de cadena ligera comprenden los siguientes elementos:

FR-L1 (DIQMTQSPSSLSASVGDRVTITC, identificador de secuencia n.º: 17);

FR-L2 (WYQQKPGKAPKLLIY, identificador de secuencia n.º: 18);

FR-L3 (GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC, identificador de secuencia n.º: 19)

FR-L4 (FGQGTKVEIKR, identificador de secuencia n.º: 20).

[0272] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena ligera que comprende una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia con respecto a una secuencia de aminoácidos del identificador de secuencia n.º: 6. En ciertas formas de realización, una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia contiene sustituciones, adiciones o eliminaciones relativas a la secuencia de

referencia; sin embargo, un anticuerpo que comprende una secuencia de aminoácidos conserva la capacidad de unirse a STEAP-1. En ciertas formas de realización, un total de entre 1 y 10 aminoácidos se han sustituido, insertado o eliminado en una secuencia seleccionada del identificador de secuencia n.º: 6, 11, 12, 13, 17, 18, 19, y 20. En ciertas formas de realización, las sustituciones, inserciones o eliminaciones se producen las regiones externas a las HVR (es decir, en las FR). En ciertas formas de realización, un anticuerpo anti-STEAP-1 comprende un dominio variable de cadena ligera que contiene la secuencia de aminoácidos del identificador de secuencia n.º: 6.

[0273] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende (a) un dominio variable de cadena pesada que comprende una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia con respecto a una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 9 y 10 y (b) un dominio variable de cadena ligera que comprende una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia con respecto a una secuencia de aminoácidos del identificador de secuencia n.º: 6. En ciertas formas de realización, una secuencia de aminoácidos con al menos un 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% o 99% de identificación de secuencia contiene sustituciones, adiciones o eliminaciones relativas a la secuencia de referencia; sin embargo, un anticuerpo que comprende esa secuencia de aminoácidos conserva la capacidad de unirse a STEAP-1. En ciertas formas de realización, un total de entre 1 y 10 aminoácidos se han sustituido, insertado o eliminado en la secuencia de referencia, incluyendo, entre otras, una secuencia seleccionada a partir del identificador de secuencia nº 9, 10, 14, 15, 16, 21, 22, 23, 24, 25, 75, 76, 77, 78, 79. En ciertas formas de realización, las sustituciones, inserciones o eliminaciones se producen en regiones externas a las HVR (es decir, en las FR). En ciertas formas de realización, un anticuerpo anti-STEAP-1 comprende un dominio variable de cadena pesada que comprende una secuencia de aminoácidos del identificador de secuencia n.º: 9 y 10 y un dominio variable de cadena ligera que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 6.

[0274] En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende (a) una, dos o tres HVR de VH seleccionadas de las que se muestran en la Figura 2B o (b) una, dos o tres HVR de VL seleccionadas de las que se muestran en la Figura 2A. En un aspecto, la invención proporciona un anticuerpo anti-STEAP-1 que comprende un dominio variable de cadena pesada seleccionado entre los que se muestran en la Figura 2B y un dominio variable de cadena ligera seleccionado entre los que se muestran en la Figura 2A.

2. Fragmentos de anticuerpos

[0275] La presente invención comprende fragmentos de anticuerpos. Los fragmentos de anticuerpos se pueden generar por métodos tradicionales, como digestión enzimática, o mediante técnicas

recombinantes. En determinadas circunstancias, resulta útil utilizar fragmentos de anticuerpos en vez de anticuerpos completos. El menor tamaño de los fragmentos permite una rápida eliminación y puede brindar un mejor acceso a los tumores sólidos. Para obtener una revisión de determinados fragmentos de anticuerpos, véase Hudson y col. (2003) *Nat. Med.* 9:129-134.

[0276] Se han desarrollado varias técnicas para la producción de fragmentos de anticuerpos. Tradicionalmente, estos fragmentos se obtenían mediante digestión proteolítica de anticuerpos intactos (véase, por ejemplo, Morimoto y col., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) y Brennan y col., *Science*, 229:81 (1985)). Sin embargo, estos fragmentos ahora pueden producirse directamente mediante células hospederas recombinantes. Los fragmentos de anticuerpos Fab, Fv y ScFv pueden expresarse todos en *E. coli* y secretarse a partir de *E. coli*, lo que permite la fácil producción de grandes cantidades de estos fragmentos. Los fragmentos de anticuerpos se pueden aislar de las bibliotecas de fagos descritas anteriormente. De manera alternativa, los fragmentos Fab'-SH pueden recuperarse directamente de *E. coli* y unirse químicamente para formar fragmentos $F(ab')_2$ (Carter y col., *Bio/Technology* 10:163-167 (1992)). Según otro método, los fragmentos $F(ab')_2$ se pueden aislar directamente desde cultivos de células hospederas recombinantes. El Fab y el fragmento $F(ab')_2$ con mayor semivida *in vivo* que comprende residuos de un epítopo de unión a receptor de rescate se describe en la patente de EE.UU. N.º 5.869.046. Otras técnicas para la producción de fragmentos de anticuerpos serán conocidas por los expertos en la materia. En ciertas formas de realización, un anticuerpo es un fragmento Fv de cadena única (scFv). Véase el documento WO 93/16185; la patente de EE.UU. N.º 5.571.894 y la patente de EE.UU. N.º 5.587.458. Fv y scFv son las únicas especies con puntos de combinación intactos que están privados de regiones constantes; por ello, pueden ser aptos para una unión no específica reducida durante el uso *in vivo*. Las proteínas de fusión scFv se pueden construir para obtener la fusión de una proteína efectora en el extremo carboxi terminal o amino terminal de un scFv. Véase *Antibody Engineering*, ed. Borrebaeck, mencionado anteriormente. El fragmento de anticuerpo puede ser también un "anticuerpo lineal", por ejemplo, tal y como se describe en la patente de EE.UU. N.º 5.641.870. Estos anticuerpos lineales pueden ser monoespecíficos o biespecíficos.

3. Anticuerpos humanizados

[0277] La presente invención comprende anticuerpos humanizados. En la materia se conocen diversos métodos para humanizar anticuerpos no humanos. Por ejemplo, un anticuerpo humanizado puede tener uno o más residuos de aminoácidos introducidos en él desde una fuente que sea no humana. Estos residuos de aminoácidos no humanos se conocen a menudo como residuos "importados", que generalmente se toman de un dominio variable de "importación". La humanización se puede realizar esencialmente siguiendo el método de Winter y sus colaboradores (Jones y col., (1986) *Nature* 321:522-525; Riechmann y col. (1988) *Nature* 332:323-327; Verhoeyen y col. (1988) *Science* 239:1534-1536), sustituyendo las

secuencias de la región hipervariable por las secuencias correspondientes de un anticuerpo humano. Por consiguiente, dichos anticuerpos "humanizados" son anticuerpos quiméricos (patente de EE.UU. Nº 4.816.567) en donde se ha sustituido sustancialmente menos de un dominio variable humano intacto por la secuencia correspondiente de especies no humanas. En la práctica, los anticuerpos humanizados son generalmente anticuerpos humanos en los que los residuos de la región hipervariable y posiblemente algunos residuos de la FR se sustituyen por residuos de sitios análogos en los anticuerpos de roedores.

[0278] La elección de los dominios variables humanos, tanto ligeros como pesados, para realizar los anticuerpos humanizados puede ser muy importante para disminuir la antigenicidad. De acuerdo con el llamado método del "más apto", la secuencia del dominio variable de un anticuerpo de un roedor se compara con toda la bliblioteca conocida de secuencias de dominio variable de humanos. Entonces, se acepta la secuencia humana más parecida a la de los roedores como estructura humana para el anticuerpo humanizado (Sims y col. (1993) *J. Immunol.* 151:2296; Chothia y col. (1987) *J. Mol. Biol.* 196:901. Otro método utiliza una estructura concreta derivada de la secuencia de consenso de todos los anticuerpos humanos de un subgrupo en particular de cadenas ligeras o pesadas. Esta misma estructura se puede usar para varios anticuerpos humanizados diferentes (Carter y col. (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta y col. (1993) *J. Immunol.*, 151:2623.

[0279] Es deseable también, por lo general, que los anticuerpos se humanicen con retención de alta afinidad para el antígeno y otras propiedades biológicas favorables. Para lograr este objetivo, siguiendo uno de los métodos, los anticuerpos humanizados se preparan mediante un proceso de análisis de las secuencias parentales y varios productos humanizados conceptuales por medio de modelos tridimensionales de las secuencias parentales y humanizadas. Generalmente se dispone de modelos de inmunoglobina tridimensional conocidos por los expertos en la materia. Existen programas informáticos que ilustran y muestran estructuras conformacionales tridimensionales probables de secuencias de inmunoglobulina candidata. La inspección de estas visualizaciones posibilita el análisis de la función probable de los residuos en el funcionamiento de la secuencia de inmunoglobulina candidata, es decir, el análisis de aquellos residuos que influyen en la capacidad de la inmunoglobulina candidata para unirse a su antígeno. De esta manera, los residuos de la FR se pueden seleccionar y combinar a partir de las secuencias receptoras y de importación para lograr la característica deseada del anticuerpo, como por ejemplo, una mayor afinidad para el/los antígeno/s diana. En general, los residuos de la región hipervariable están directamente y más considerablemente involucrados en la influencia que se ejerce sobre la unión a antígeno.

4. Anticuerpos humanos

[0280] Los anticuerpos humanos anti-STEAP-1 de la invención se pueden construir combinando una o más secuencias de dominio variable Fv de clones seleccionadas de bibliotecas de representación en imágenes de fagos derivados de humanos con una o más secuencias de domino constante humanas, como

se ha descrito anteriormente. Alternativamente, los anticuerpos anti-STEAP-1 monoclonales humanos de la invención se pueden producir mediante el método del hibridoma. Las líneas celulares del mieloma humano y heteromieloma humano-murino también se han descrito para la producción de anticuerpos monoclonales humanos, por ejemplo, Kozbor, *J. Immunol.*, 133: 3001 (1984); Brodeur y col., *Monoclonal Antibody Production Techniques and Applications*, págs. 51-63 (Marcel Dekker, Inc., New York, 1987) y Boerner y col, *J. Immunol.*, 147: 86 (1991).

[0281] Actualmente es posible producir animales transgénicos (por ejemplo, ratones) que sean capaces, una vez inmunizados, de generar un repertorio completo de anticuerpos humanos cuando no exista producción de inmunoglobulina endógena. Por ejemplo, se ha descrito que la eliminación homocigótica del gen de la región de unión (JH) de la cadena pesada del anticuerpo en ratones quiméricos y mutantes de línea germinal ocasiona la inhibición total de la producción de anticuerpos endógenos. La transferencia de la matriz de genes humanos de la inmunoglobulina de línea germinal en uno de estos ratones mutantes de línea germinal ocasionará la producción de anticuerpos humanos al actuar sobre el antígeno. Véase, por ejemplo, Jakobovits y col., *Proc. Natl. Acad. Sci USA,* 90: 2551 (1993); Jakobovits y col., *Nature,* 362: 255 (1993); Bruggermann y col., *Year in Immunol.*, 7: 33 (1993).

[0282] También se puede utilizar la combinación de genes para derivar anticuerpos humanos de no humanos, por ejemplo, anticuerpos de roedores, en los que el anticuerpo humano tiene afinidades y especificidades similares a las del anticuerpo no humano de partida. Según este método, que también se denomina "impronta de epítopos", la región variable de cadena pesada o ligera de un fragmento de anticuerpo no humano obtenido mediante técnicas de representación en imágenes de fagos, como las descritas anteriormente, se sustituye por un repertorio de genes de dominio V humanos, lo que crea una población de quimeras Fab o scFv de cadena humana/cadena no humana. La selección con el antígeno da lugar al aislamiento de un Fab o scFV quimérico de cadena humana/cadena no humana, en el que la cadena humana restaura el sitio de unión a antígeno destruido al eliminar la cadena no humana correspondiente del clon de representación de fagos primario; es decir, el epítopo rige (impregna) la elección de la molécula de la cadena humana. Cuando se repite el proceso para sustituir la cadena no humana restante, se obtiene un anticuerpo humano (véase el documento PCT WO 93/06213 publicado el 1 de abril de 1993). A diferencia de la tradicional humanización de anticuerpos no humanos mediante transplante de CDR, esta técnica proporciona anticuerpos completamente humanos que no tienen residuos de la FR o CDR de origen no humano.

5. Anticuerpos biespecíficos

[0283] Los anticuerpos biespecíficos son anticuerpos monoclonales que tienen especificidades de unión con al menos dos antígenos diferentes. En ciertas formas de realización, los anticuerpos biespecíficos son anticuerpos humanos o humanizados. En ciertas formas de realización, una de las

especificidades de unión corresponde a STEAP-1 y la otra, a cualquier otro antígeno. En ciertas formas de realización, los anticuerpos biespecíficos se pueden unir a dos epítopos diferentes de STEAP-1. Los anticuerpos biespecíficos también se pueden utilizar para localizar agentes citotóxicos de las células que expresan el STEAP-1. Estos anticuerpos poseen un brazo de unión a STEAP-1 y un brazo que se une a un agente citotóxico, como por ejemplo, saporina, anti-interferón-α, alcaloide de la vinca, cadena de ricina A, metotrexato o hapteno con un isótopo radiactivo. Los anticuerpos biespecíficos se pueden preparar como anticuerpos de longitud completa o fragmentos de anticuerpos (por ejemplo, anticuerpos biespecíficos $F(ab')_2$).

[0284] Los procedimientos para realizar anticuerpos biespecíficos son conocidos en la materia. La producción recombinante tradicional de anticuerpos biespecíficos se basa en la coexpresión de dos pares de cadena pesada-cadena ligera de inmunoglobulina, en donde las dos cadenas tienen distintas especificidades (Milstein y Cuello, *Nature*, 305: 537 (1983)). Debido a la variedad aleatoria delas cadenas pesadas y ligeras de la inmunoglobulina, estos hibridomas (células híbridas) producen una posible mezcla de 10 moléculas diferentes de anticuerpos, de las cuales sólo una tiene la estructura biespecífica correcta. La purificación de la molécula correcta, que generalmente se realiza mediante etapas de cromatografía de afinidad, es bastante complicada, y la cantidad de producto que se obtiene es baja. Se describen procedimientos similares en el documento WO 93/08829 publicado el 13 de mayo de 1993 y en Traunecker y col., *EMBO J.*, 10: 3655 (1991).

[0285] Según un método distinto, los dominios variables de anticuerpos con las especificidades de unión deseadas (puntos de combinación anticuerpo-antígeno) se fusionan con las secuencias de domino constante de inmunoglobulina. La fusión se realiza, por ejemplo, con un dominio constante de la cadena pesada de la inmunoglobulina, que comprende al menos parte de las regiones bisagra, CH2 y CH3. En ciertas formas de realización, la primera región constante de la cadena pesada (CH1), que contiene el lugar necesario para la unión de la cadena ligera, está presente en al menos una de las fusiones. Los ADN que codifican las fusiones de la cadena pesada de la inmunoglobulina y, si se desea, de la cadena ligera de la inmunoglobulina, se insertan en vectores de expresión separados y se cotransfectan a un organismo hospedero adecuado. Esto proporciona mayor flexibilidad al ajustar las proporciones mutuas de los tres fragmentos de polipéptidos en formas de realización en donde las proporciones desiguales de las tres cadenas de polipéptidos utilizados en la construcción proporcionan rendimientos óptimos. Sin embargo, es posible insertar las secuencias de codificación de dos o de las tres cadenas de polipéptidos en un vector de expresión cuando la expresión de al menos dos cadenas de polipéptidos con proporciones iguales genera rendimientos altos o cuando las proporciones no tienen particular importancia.

[0286] En una forma de realización de este método, los anticuerpos biespecíficos se componen de una cadena pesada de inmunoglobulina híbrida con una primera especificidad de unión en un brazo y un

par de cadena pesada-cadena ligera de inmunoglobulina híbrida (que proporciona una segunda especificidad de unión) en el otro brazo. Se descubrió que esta estructura asimétrica facilita la separación del compuesto biespecífico deseado de las combinaciones de cadenas de inmunoglobulina no deseadas, ya que la presencia de una cadena ligera de inmunoglobulina en sólo una de las mitades de la molécula biespecífica proporciona una forma sencilla de separación. Este método se desveló en el documento WO 94/04690. Para obtener mayores detalles sobre la generación de anticuerpos biespecíficos, véase, por ejemplo, Suresh y col., *Methods in Enzymology*, 121:210 (1986).

[0287] Según otro método, la interfaz entre un par de moléculas de anticuerpos se puede modificar para maximizar el porcentaje de heterodímeros que se recuperan de un cultivo de células recombinantes. La interfaz comprende al menos una parte del dominio C_H3 de un dominio constante de anticuerpos. En este método, una o más cadenas laterales cortas de aminoácidos de la interfaz de la primera molécula del anticuerpo se reemplazan con cadenas laterales más largas (por ejemplo, tirosina o triptofano). Las "cavidades" compensatorias de tamaño idéntico o similar a la(s) cadena(s) lateral(es) larga(s) se crean en la interfaz de la segunda molécula del anticuerpo al reemplazar las cadenas laterales largas de aminoácidos por cadenas más cortas (por ejemplo alanina o treonina). Esto proporciona un mecanismo para incrementar la producción del heterodímero con relación a otros productos finales no deseados como los homodímeros.

[0288] Los anticuerpos biespecíficos incluyen anticuerpos reticulantes o "heteroconjugados". Por ejemplo, uno de los anticuerpos en el heteroconjugado se puede unir a la avidina y el otro a la biotina. Estos anticuerpos se han propuesto, por ejemplo, para conseguir que las células del sistema inmunitario actúen sobre células no deseadas (patente de EE. UU. N.º 4.676.980) y para el tratamiento de la infección de VIH (documentos WO 91/00360 y WO 92/00373, y la patente europea EP 03089). Los anticuerpos heteroconjugados pueden obtenerse utilizando cualquier método reticulante conveniente. Los agentes reticulantes adecuados se conocen bien en la materia y se desvelan en la patente de EE. UU. N.º 4.676.980, junto con una serie de técnicas de reticulación.

[0289] Las técnicas para generar anticuerpos biespecíficos a partir de fragmentos de anticuerpos también se han descrito en la bibliografía. Por ejemplo, los anticuerpos biespecíficos se pueden preparar mediante un enlace químico. Brennan y col., *Science*, 229: 81 (1985) describe un procedimiento en el que los anticuerpos intactos se separan proteolíticamente para generar fragmentos $F(ab')_2$. Estos fragmentos se reducen en presencia de arsenito de sodio del agente que forma complejos con el ditiol para estabilizar los ditioles vecinales y evitar la formación de disulfuros intermoleculares. Los fragmentos Fab' generados posteriormente se convierten en derivados del tionitrobenzoato (TNB). Uno de los derivados del Fab'-TNB posteriormente se reconvierte en Fab'-tiol por reducción con mercaptoetilamina y se mezcla con una cantidad equimolar de otro derivado del Fab'-TNB para formar el anticuerpo biespecífico. Los anticuerpos biespecíficos producidos se pueden utilizar como agentes para la inmovilización selectiva de enzimas.

[0290] Descubrimientos actuales han facilitado la recuperación directa de fragmentos Fab'-SH de *E. coli*, que se pueden unir químicamente para formar anticuerpos biespecíficos. Shalaby y col., *J. Exp. Med.*, 175: 217-225 (1992) describe la producción de una molécula $F(ab')_2$ de anticuerpo biespecífico completamente humanizado. Cada fragmento Fab' se secretó por separado a partir de *E. coli* y se sometió a un emparejamiento químico dirigido *in vitro* para formar el anticuerpo biespecífico. Por lo tanto, el anticuerpo biespecífico formado era capaz de unirse a células que sobreexpresaban el receptor HER2 y las células T humanas normales, así como de desencadenar la actividad lítica de los linfocitos citotóxicos humanos contra dianas de tumor de mama humano.

[0291] También se han descrito varias técnicas para realizar y aislar fragmentos de anticuerpos biespecíficos directamente de cultivos de células recombinantes. Por ejemplo, se han producido anticuerpos biespecíficos utilizando cremalleras de leucina. Kostelny y col., *J. Immunol.*, 148(5):1547-1553 (1992). Los péptidos con cremallera de leucina de las proteínas Fos y Jun se unieron a las porciones Fab' de dos anticuerpos diferentes mediante fusión génica. Los homodímeros del anticuerpo se redujeron en la región bisagra para formar monómeros y después se reoxidaron para formar los heterodímeros del anticuerpo. Este método también se puede utilizar para producir los homodímeros del anticuerpo. La tecnología "diacuerpo" descrita por Hollinger y col., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) ha proporcionado un mecanismo alternativo para realizar fragmentos de anticuerpos biespecíficos. Los fragmentos comprenden un dominio variable de cadena pesada (VH) conectado a un dominio variable de cadena ligera (VL) mediante un enlazador que es demasiado corto para permitir el emparejamiento entre los dos dominios de la misma cadena. Por consiguiente, los dominios VH y VL de un fragmento se fuerzan para emparejarse con los dominios VL y VH complementarios de otro fragmento; por lo tanto, forman dos sitios de unión al antígeno. También se ha informado de otra estrategia para realizar fragmentos de anticuerpos biespecíficos mediante el uso de dímeros Fv de cadena única (sFv). Véase Gruber y col., *J. Immunol.*, 152:5368 (1994).

[0292] También se contemplan los anticuerpos con más de dos valencias. Por ejemplo, se pueden preparar anticuerpos triespecíficos. Tutt y col., *J. Immunol.* 147: 60 (1991).

6. Anticuerpos multivalentes

[0293] Un anticuerpo multivalente puede internalizarse (y/o catabolizarse) más rápido que un anticuerpo bivalente por una célula que exprese un antígeno al que se unen los anticuerpos. Los anticuerpos de la presente invención pueden ser anticuerpos multivalentes (que son los que no pertenecen a la clase IgM) con tres o más sitios de unión al antígeno (por ejemplo, anticuerpos tetravalentes), que pueden producirse fácilmente mediante la expresión recombinante del ácido nucleico que codifica las cadenas de polipéptidos del anticuerpo. El anticuerpo multivalente puede comprender un dominio de dimerización y tres o más sitios de unión al antígeno. En ciertas formas de realización, el dominio de dimerización

comprende (o está formado por) una región Fc o una región bisagra. En esta situación, el anticuerpo comprenderá una región Fc y tres o más sitios de unión amino-terminal del antígeno a la región Fc. En ciertas formas realización, un anticuerpo multivalente comprende (o está formado por) aproximadamente entre tres y ocho sitios de unión a antígeno. En una de estas formas de realización, un anticuerpo multivalente comprende (o está formado por) cuatro sitios de unión a antígeno. El anticuerpo multivalente comprende al menos una cadena de polipéptidos (por ejemplo, dos cadenas de polipéptidos) en la que la(s) cadena(s) de polipéptidos comprende(n) dos o más dominios variables. Por ejemplo, la(s) cadena(s) de polipéptidos puede(n) comprender VD1-(X1)n-VD2-(X2)n-Fc, donde VD1 es un primer dominio variable, VD2 es un segundo dominio variable, Fc es una cadena de polipéptidos de una región Fc, X1 y X2 representan una aminoácido o polipéptido y n es 0 ó 1. Por ejemplo, la(s) cadena(s) de polipéptidos puede(n) comprender: VH-CH1-enlazador flexible-VH-CH1- cadena de la región FC o VH-CH1-VH-CH1-cadena de la región Fc. El anticuerpo multivalente de la presente memoria descriptiva puede comprender, además, al menos dos (por ejemplo, cuatro) polipéptidos de dominio variable de cadena ligera. El anticuerpo multivalente de la presente memoria descriptiva puede, por ejemplo, comprender entre dos y ocho polipéptidos de dominio variable de cadena ligera. Los polipéptidos de dominio variable de cadena ligera considerados en esta memoria descriptiva comprenden un dominio variable de cadena ligera y, opcionalmente, un dominio CL.

7. Anticuerpos de dominio único

[0294] En ciertas formas de realización, un anticuerpo de la invención es un anticuerpo de dominio único. Un anticuerpo de dominio único es una única cadena polipeptídica que comprende la totalidad o parte del dominio variable de la cadena pesada o la totalidad o parte del dominio variable de la cadena ligera de un anticuerpo. En ciertas formas de realización, un anticuerpo de dominio único es un anticuerpo humano de dominio único (Domantis, Inc., Waltham, MA; véase, por ejemplo, patente de EE. UU. N.º 6.248.516 B1). En una forma de realización, un anticuerpo de dominio único está formado por la totalidad o parte del dominio variable de la cadena pesada de un anticuerpo.

8. Variantes de anticuerpos

[0295] En ciertas formas de realización, se contemplan la(s) modificación(es) de la secuencia de aminoácidos descrita(s) en la presente memoria descriptiva. Por ejemplo, puede desearse mejorar la afinidad de unión u otras propiedades biológicas del anticuerpo. Las variantes de la secuencia de aminoácidos del anticuerpo se pueden preparar mediante la introduccción de cambios adecuados en la secuencia de nucleótidos que codifica el anticuerpo o mediante la síntesis de péptidos. Dichas modificaciones incluyen, por ejemplo, eliminaciones de o inserciones dentro o sustituciones de residuos dentro de las secuencias del aminoácido del anticuerpo. Se puede realizar cualquier combinación de eliminación, inserción y sustitución para llegar a la construcción final, siempre que ésta posea las

características deseadas. Las alteraciones aminoacídicas pueden introducirse en la secuencia de aminoácidos del anticuerpo del sujeto en el momento en que se realiza la secuencia.

[0296] Un método útil para la identificación de determinados residuos o regiones del anticuerpo que constituyen ubicaciones preferidas para la mutagénesis se denomina "mutagénesis de barrido de alanita", tal como se describe en Cunningham y Wells (1989) *Science*, 244:1081-1085. En la presente memoria descriptiva, se identifica un residuo o grupo de residuos de dianas (por ejemplo, residuos cargados como arg, asp, his, lys y glu) y se sustituyen por un aminoácido con carga negativa o neutra (alanina o polialanina) para afectar la interacción de los aminoácidos con el antígeno. Estas localizaciones de los aminoácidos que demuestran sensibilidad funcional a las sustituciones se refinan al introducir más u otras variantes en, o para, los sitios de sustitución. Por lo tanto, a pesar de que el sitio donde se introducirá una variación de una secuencia de aminoácidos está predeterminado, no es necesario que la naturaleza de la mutación per se tenga un carácter predeterminado. Por ejemplo, para analizar el rendimiento de una mutación en un lugar determinado, se realiza el barrido de alanina o mutagénesis aleatoria en el codón o región diana y las inmunoglobulinas expresadas se monitorizan para detectar la actividad deseada.

[0297] Las inserciones de la secuencia de aminoácidos incluyen fusiones en los extremos amino o carboxi-terminal que abarcan desde un residuo hasta polipéptidos que contienen cien o más residuos, así como inserciones en la secuencia de uno o múltiples residuos de aminoácidos. Entre los ejemplos de inserciones terminales se encuentra un anticuerpo con un residuo metionil en el N-terminal. Otras variantes de inserción de la molécula del anticuerpo incluyen la fusión del N-terminal o C del anticuerpo con una enzima (por ejemplo, para ADEPT) o un polipéptido que aumente la semivida sérica del anticuerpo.

[0298] En ciertas formas de realización, un anticuerpo de la invención se altera para aumentar o reducir la glicosilación del anticuerpo. La glicosilación de los polipéptidos se realiza generalmente por enlace N u O. El enlace N se refiere a la unión de una fracción glucídica a la cadena lateral de un residuo de asparragina. Las secuencias de tripéptidos asparragina-X-serina y asparragina-X-treonina, en donde X equivale a cualquier aminoácido excepto la prolina, son las secuencias de reconocimiento para las uniones enzimáticas del residuo glucídico a la cadena lateral de asparragina. Por lo tanto, la presencia de cualquiera de estas secuencias de tripéptidos en un polipéptido crea un lugar de glicosilación potencial. La glicosilación por enlace O hace referencia a la unión de uno de los azúcares N-acetilgalactosamina, galactosa o xilosa a un hidroxiaminoácido, más comúnmente serina o treonina, a pesar de que también se puede utilizar 5-hidroxiprolina y 5-hidroxilisina.

[0299] La adición o eliminación de sitios de glicosilación al anticuerpo se logra convenientemente al alterar la secuencia del aminoácido para que se cree o elimine una o más de las secuencias de tripéptidos descritas anteriormente (para sitios de glicosilación unidos por enlace N). La alteración también se puede realizar mediante la adición de uno o más residuos de serina o treonina a la secuencia del anticuerpo

original (para sitios de glicosilación unidos por enlace tipo O), o la eliminación de estos residuos o su sustitución.

[0300] Donde el anticuerpo comprende una región Fc, se puede alterar el carbohidrato unido al mismo. Por ejemplo, los anticuerpos con una estructura de carbohidratos madura que carece de fucosa unida a una región Fc del anticuerpo se describen en la Solicitud de patente de EE.UU. N.º 2003/0157108 (Presta, L.). Véase también patente de EE.UU. N.º 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Los anticuerpos con una N-acetilglucosamina (GlcNAc) bisectriz en el carbohidrato unido a una región Fc del anticuerpo se describen en el documento WO 2003/011878, Jean-Mairet y col. y en la patente de EE.UU. N.º 6.602.684, Umaña y col. Los anticuerpos con al menos un residuo de galactosa en el oligosacárido unido a una región Fc del anticuerpo se describen en el documento WO 1997/30087, Patel y col. Véanse también los documentos WO 1998/58964 (Raju, S.) y WO 1999/22764 (Raju, S.) con respecto a los anticuerpos con carbohidratos alterados unidos a la región Fc de los mismos. Véase también la patente de EE.UU. 2005/0123546 (Umana y col.) sobre moléculas de unión a antígeno con glicosilación modificada.

[0301] En ciertas formas de realización, una variante de glicosilación comprende una región Fc, en la que una estructura de carbohidratos anexa a la región Fc carece de fucosa. Estas variantes han mejorado la función de la ADCC. Opcionalmente, la región Fc también comprende una o más sustituciones de aminoácidos en la zona que mejora aún más la ADCC; por ejemplo, sustituciones en las posiciones 298, 333 o 334, o todas ellas, de la región Fc (numeración Eu de residuos). Entre las publicaciones relacionadas con los anticuerpos "defucosilados" o "con déficit de fucosa" podríamos citar: la patente de EE.UU. N.º 2003/0157108; el documento WO 2000/61739; el documento WO 2001/29246; la patente de EE.UU. N.º 2003/0115614; la patente de EE.UU. N.º 2002/0164328; la patente de EE.UU. N.º 2004/0093621; la patente de EE.UU. N.º 2004/0132140; la patente de EE.UU. N.º 2004/0110704; la patente de EE.UU. N.º 2004/0110282; la patente de EE.UU. N.º 2004/0109865; el documento WO 2003/085119; el documento WO 2003/084570; el documento WO 2005/035586; el documento WO 2005/035778; el documento WO2005/053742; Okazaki y col. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki y col. *Biotech. Bioeng.* 87: 614 (2004). Entre los ejemplos de líneas celulares que producen anticuerpos defucosilados encontraríamos las células Lec13 de ovario de hámster chino (CHO) con déficit de fucosilación de proteínas (Ripka y col., *Arch. Biochem. Biophys.* 249:533-545 (1986); la Solicitud de patente de EE. UU. N.º US 2003/0157108 A1, Presta, L y el documento WO 2004/056312 A1, Adams y col, especialmente el ejemplo 11) y las líneas celulares bloqueadas, como el gen alfa-1,6-fucosiltransferasa, el FUT8 y células de ovario de hámster chino (CHO) bloqueadas (Yamane-Ohnuki y col., *Biotech. Bioeng.* 87: 614 (2004)).

[0302] En una forma de realización, el anticuerpo se modifica para mejorar su semivida sérica. A fin de incrementar la semivida sérica del anticuerpo, se le puede incorporar un epítopo de unión a receptor de rescate (especialmente un fragmento de anticuerpo) tal como se describe en la patente de EE.UU. N.º

5739277, por ejemplo. Tal como se utiliza en la presente memoria descriptiva, el término "epítopo de unión a receptor de rescate" hace referencia a un epítopo de la región Fc de una molécula de IgG (por ejemplo, IgG_1, IgG_2, IgG_3 o IgG_4) que es responsable de incrementar la semivida sérica *in vivo* de la molécula de IgG (patente de EE.UU. N.º 2003/0190311, patente de EE.UU. N.º 6821505; patente de EE.UU. N.º 6165745; patente de EE.UU. N.º 5624821; patente de EE.UU. N.º 5648260; patente de EE.UU. N.º 6165745; patente de EE.UU. N.º 5834 597).

[0303] Otro tipo de variante es una variante de sustitución aminoacídica. Estas variantes reemplazan al menos un residuo de aminoácidos en la molécula del anticuerpo por un residuo diferente. Los sitios de mayor interés para la mutagénesis por sustitución incluyen las regiones hipervariables, pero también se contemplan las alteraciones FR. Las sustituciones conservadoras se muestran en la Tabla 1 en el título "sustituciones preferentes". Si dichas sustituciones ocasionan un cambio deseable en la actividad biológica, entonces los productos se pueden examinar y se pueden introducir cambios más sustanciales, denominados "sustituciones ejemplares" en la Tabla 1, o como se describe con más detalle a continuación con referencia a las clases de aminoácidos.

TABLA 1

Residuo original	**Sustituciones preferentes**	**Sustituciones preferentes**
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lis; Gln; Asn	Lis
Asn (N)	Gln; His; Asp, Lis; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cis (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gli (G)	Ala	Ala
His (H)	Asn; Gln; Lis; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Fe; Norleucina	Leu
Leu (L)	Norleucina; Ile; Val; Met; Ala; Fe	Ile
Lis (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Fe; Ile	Leu
Fe (F)	Trp; Leu; Val; Ile; Ala; Tir	Tir
Pro (P)	Ala	Ala

Residuo original	Sustituciones preferentes	Sustituciones preferentes
Ser (S)	Tr	Tr
Tr (T)	Val; Ser	Ser
Trp (W)	Tir; Fe	Tir
Tir (Y)	Trp; Fe; Tr; Ser	Fe
Val (V)	Ile; Leu; Met; Fe; Ala; Norleucina	Leu

[0304] Las modificaciones sustanciales en las propiedades biológicas del anticuerpo se logran seleccionando sustituciones que difieran significativamente en su efecto de mantener (a) la estructura de la base del polipéptido en el área de la sustitución, por ejemplo, como una conformación plana o helicoidal, (b) la carga o hidrofobicidad de la molécula en el lugar diana, o (c) el residuo de la cadena lateral. Los aminoácidos pueden agruparse de acuerdo con similitudes en las propiedades de sus cadenas laterales (en A. L. Lehninger, en *Biochemistry*, segunda ed., págs. 73-75, Worth Publishers, Nueva York (1975)):

(1) no polares: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) polares no cargados: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) ácidos: Asp (D), Glu (E)

(4) básicos: Lys (K), Arg (R), His (H)

[0305] Alternativamente, los residuos que se obtienen naturalmente pueden dividirse en grupos en base a las propiedades comunes de las cadenas laterales:

(1) hidrófobos: Norleucina, Met, Ala, Val, Leu, Ile;

(2) hidrófilos neutrales: Cis, Ser, Tr, Asn, Gln;

(3) ácidos: Asp, Glu;

(4) básicos: His, Lys, Arg;

(5) residuos que tiene influencia en la orientación de la cadena: Gly, Pro;

(6) aromáticos: Trp, Tyr, Phe.

[0306] Las sustituciones no conservadoras supondrán intercambiar un miembro de una de estas clases por otra clase. Estos residuos sustituidos también se pueden introducir en sitios de sustitución conservadora o en los sitios restantes (no conservados).

[0307] Un tipo de variante de sustitución implica sustituir uno o más residuos de región hipervariable de un anticuerpo parental (por ejemplo, un anticuerpo humanizado o humano). Por lo general, la(s) variante(s) resultante(s) seleccionada(s) para profundizar en su desarrollo tendrá(n) propiedades biológicas modificadas (es decir, mejoradas) con relación al anticuerpo parental desde el cual se generó (generaron). Una manera conveniente de generar dichas variantes de sustitución implica la maduración de afinidad utilizando una presentación en fagos. De manera resumida, varios sitios de regiones hipervariables (por ejemplo, 6-7 sitios) se mutan para generar todas las sustituciones posibles de

aminoácidos en cada sitio. Los anticuerpos así generados se representan a partir de partículas de fagos filamentosos como fusiones a, al menos, parte de una proteína de revestimiento de fagos (por ejemplo, con el producto del gen III de M13) que hay dentro de cada partícula. Las variantes presentadas en fagos se monitorizan para detectar su actividad biológica (por ejemplo, afinidad de unión). Para identificar los lugares de la región hipervariable candidatos para la modificación, se puede realizar mutagenia mediante barridos (por ejemplo, barridos de alanina) para identificar residuos de la región hipervariable que contribuyan significativamente a la unión a antígeno. Como alternativa, o adicionalmente, puede ser beneficioso analizar una estructura cristalina del complejo antígeno-anticuerpo para identificar los puntos de contacto entre el anticuerpo y el antígeno. Dichos residuos de contacto y los residuos adyacentes son candidatos a la sustitución de acuerdo con las técnicas conocidas en la materia, incluidas las explicadas en la presente memoria descriptiva. Una vez generadas dichas variantes, el panel de variantes está sujeto a examen utilizando técnicas conocidas en la materia, incluidas las descritas en la presente memoria descriptiva, y los anticuerpos con propiedades superiores en uno o más ensayos pertinentes se pueden seleccionar para profundizar en su desarrollo.

[0308] Las moléculas de ácido nucleico que codifican variantes de secuencia de aminoácidos del anticuerpo se preparan mediante varios métodos conocidos en la materia. Estos métodos incluyen, entre otros, aislamiento desde una fuente natural (en el caso de variantes de secuencia de aminoácidos presentes en la naturaleza) o preparación mediante mutagénesis con mediación oligonucleótida (o controlada *in situ*), mutagénesis PCR y mutagénesis de módulo de una variante preparada anteriormente o una versión no variante del anticuerpo.

[0309] Puede ser deseable introducir una o más modificaciones en los aminoácidos en la región Fc de anticuerpos de la invención, lo que genera una variante de la región Fc. La variante de la región Fc puede comprender una secuencia de la región Fc humana (por ejemplo, una región Fc de IgG1, IgG2, IgG3 o IgG4 humanas) que contenga una modificación de aminoácidos (por ejemplo, una sustitución) en una o más posiciones de aminoácidos, incluida la de la cisteína bisagra.

[0310] Según esta descripción y los conocimientos en la materia, se contempla que en algunas realizaciones un anticuerpo de la invención pueda comprender una o más alteraciones, frente a su anticuerpo homólogo de tipo natural, por ejemplo, en la región Fc. No obstante, estos anticuerpos conservarán las mismas características necesarias para uso terapéutico, frente a su homólogo de tipo natural. Por ejemplo, se cree que se pueden realizar algunas alteraciones en la región Fc que daría como resultado una unión C1q alterada (ya sea mejorada o disminuida) o citotoxicidad dependiente de complementos (CDC), por ejemplo, como se describe en el documento WO99/51642. Véase también Duncan & Winter *Nature* 322:738-40 (1988); la patente de EE. UU. N.º 5.648.260; la patente de EE. UU. N.º 5.624.821 y el documento WO94/29351 que se refieren a otros ejemplos de variantes de regiones Fc.

Los documentos WO00/42072 (Presta) y WO 2004/056312 (Lowman) describen variantes de anticuerpos con capacidad de unión a los FcRs mejoradas o disminuidas. El contenido de estas publicaciones de patentes se incorpora específicamente a la presente memoria descriptiva a modo de referencia. Véase, además, Shields y col., *J. Biol. Chem.*, 9(2): 6591-6604 (2001). Los anticuerpos con un aumento de su semivida y una unión mejorada al receptor neonatal Fc (FcRn), que es responsable de la transmisión de las IgG maternas al feto (Guyer y col., *J. Immunol.* 117:587 (1976) y Kim y col., *J. Immunol.* 24:249 (1994)), se describen en la patente de EE.UU. N.º 2005/0014934A1 (Hinton y col.). Estos anticuerpos comprenden una región Fc con una o más sustituciones en la zona que mejoran la unión de la región Fc al FcRn. Las variantes polipeptídicas con secuencias aminoacídicas de la región Fc alteradas y capacidad aumentada o disminuida de unión a Cq1 se describen en la patente de EE.UU. N.º 6.194.551B1 y el documento WO99/51642. El contenido de dichas publicaciones de patentes se incorpora específicamente a la presente memoria descriptiva a modo de referencia. Véase, además, Idusogie y col. *J. Immunol.* 164: 4178-4184 (2000).

[0311] En un aspecto, la invención proporciona anticuerpos que comprenden modificaciones en la interfaz de polipéptidos Fc que comprenden la región Fc, donde las modificaciones facilitan y/o fomentan la heterodimerización. Estas modificaciones comprenden la introducción de una protuberancia en un primer polipéptido Fc y una cavidad en un segundo polipéptido Fc, donde la protuberancia se puede posicionar en la cavidad para que fomente la formación de complejos del primer y segundo polipéptido Fc. Los métodos de generación de anticuerpos con estas modificaciones se conocen en la materia, por ejemplo, los descritos en la patente de EE.UU. N.º 5.731.168.

9. Derivados del anticuerpo

[0312] Los anticuerpos de la presente invención pueden modificarse aún más a fin de que contengan fracciones no proteicas adicionales que son conocidas en la materia y se encuentran disponibles. Preferiblemente, las fracciones adecuadas para la derivatización del anticuerpo son polímeros solubles en agua. Ejemplos no limitantes de los polímeros solubles en agua incluyen los siguientes componentes: polietilenglicol (PEG), copolímero de etilenglicol/propilenglicol, carboximetilcelulosa, dextrano, alcohol polivinilo, polivinilpirrolidona, poli-1, 3-dioxolano, poli-1,3,6-trioxano, copolímero anhídrido etileno/maleico, poliaminoácidos (ya sean homopolímeros o copolímeros aleatorios), y dextrano o poli(n-vinilpirrolidona)polietilenglicol, homopolímeros de propropilenglicol, óxido de prolipropileno / copolímeros de óxido de etileno, polialcoholes polioxietilados (por ejemplo glicerol), alcohol polivinilo, y mezclas de los mismos. Es posible que el polietilenglicol propionaldehído ofrezca ventajas en la elaboración, dada su estabilidad en agua. El polímero puede ser de cualquier peso molecular y puede ser ramificado o no. La cantidad de polímetros adheridos al anticuerpo puede variar y, si se une más de un polímero, los polímeros pueden ser las mismas o diferentes moléculas. Generalmente, la cantidad y/o tipo

de polímeros utilizados para la derivatización pueden determinarse en función de ciertas consideraciones, como las propiedades o funciones particulares del anticuerpo a mejorar, si el anticuerpo derivatizado será utilizado en un tratamiento en condiciones definidas, etc.

[0313] En otra forma de realización, se proporcionan los conjugados del anticuerpo y la fracción no proteica que puede calentarse selectivamente mediante la exposición a radiación. En una forma de realización, la fracción no proteica es un nanotubo de carbono (Kam y col., *Proc. Natl. Acad. Sci.* 102: 11600-11605 (2005)). La radiación puede ser de cualquier longitud de onda, e incluye una longitud de onda que no dañe las celulas comunes, pero que caliente las fracciones no proteicas hasta una temperatura en la cual se eliminen las células próximas a las fracciones no proteicas del anticuerpo.

Algunos métodos de elaboración de anticuerpos

1. Algunos métodos de hibridoma

[0314] Pueden conseguirse los anticuerpos monoclonales anti-STEAP-1 de la invención por el método del hibridoma, descrito por primera vez en Kohler y col., *Nature,* 256:495 (1975) o con el método del ADN recombinante (patente de EE. UU. N.º 4.816.567).

[0315] En el método del hibridoma, un ratón u otro animal hospedero apropiado, como un hámster, se inmuniza para obtener linfocitos que produzcan o sean capaces de producir anticuerpos que se unirán específicamente a la proteína utilizada para la inmunización. Los anticuerpos anti-STEAP-1 se producen generalmente en animales mediante inyecciones subcutáneas (sc) o intraperitonales (ip) de STEAP-1 y un adyuvante. El STEAP-1 se puede preparar usando métodos bien conocidos en la materia, algunos de los cuales se describen con más detalle en la presente memoria descriptiva. Por ejemplo, el STEAP-1 se puede producir de forma recombinante. En una forma de realización, se inmuniza a los animales con un derivado de STEAP-1 que contiene una porción extracelular de STEAP-1 fusionado a la porción Fc de una cadena pesada de inmunoglobulina. En una forma de realización, se inmuniza a los animales con una proteína de fusión STEAP-1-IgG1. En una forma de realización, se inmuniza a animales con derivados inmunogénicos de STEAP-1 en una solución con monofosforil lípido A (MPL)/dicrinomicolato de trehalosa (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT) y la solución se inyecta intradérmicamente en múltiples puntos. Dos semanas más tarde los animales reciben dosis de refuerzo. Entre siete y catorce días más tarde, se les extrae sangre a los animales y el suero se analiza para titular los anticuerpos anti-STEAP-1. Los animales reciben dosis de refuerzo hasta obtener los niveles adecuados de titulación.

[0316] De manera alternativa, los linfocitos se pueden inmunizar *in vitro.* Después, los linfocitos se fusionan con las células de mieloma utilizando un agente fusionante adecuado, como por ejemplo

polietilenglicol, para formar una célula de hibridoma (Goding, *Monoclonal Antibodies: Principles and Practice*, págs. 59-103 (Academic Press, 1986)).

[0317] Por lo tanto, las células de hibridoma preparadas se siembran y cultivan en un medio de cultivo adecuado, por ejemplo, un medio que contenga una o más sustancias que inhiban el crecimiento o la supervivencia de las células de mieloma parental no fusionadas. Por ejemplo, si las células de mieloma parentales no poseen la enzima hipoxantina-guanina-fosforribosil-transferasa (HGPRT o HPRT), el medio de cultivo para los hibridomas generalmente incluirá hipoxantina, aminopterina y timidina (medio HAT), cuyas sustancias evitan el crecimiento de células con déficit de HGPRT.

[0318] En ciertas formas de realización, las células de mieloma son aquellas que se fusionan eficazmente, soportan altos niveles estables de producción de anticuerpos por las células productoras de anticuerpos seleccionadas y son sensibles a un medio como el medio HAT. Entre las células de mieloma ejemplares se encuentran, entre otras, las líneas de mieloma murino, como aquellas derivadas de los tumores de ratones MOPC-21 y MPC-11, disponibles en Salk Institute Cell Distribution Center, San Diego, California, EE.UU., y las células SP-2 o X63-Ag8-653, disponibles en el American Type Culture Collection, Rockville, Maryland, EE.UU. Las líneas celulares de mieloma humano y heteromieloma de ratón-humano también se han descrito para la producción de anticuerpos monoclonales humanos (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur y col., *Monoclonal Antibody Production Techniques and Applications*, págs. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0319] El medio de cultivo en el cual las células de hibridoma crecen se analiza para la producción de anticuerpos monoclonales que se unan a STEAP-1. Preferentemente, la especificidad de unión de los anticuerpos monoclonales producidos por las células de hibridoma está determinada por la inmunoprecipitación o por un ensayo de unión, como el radioinmunoensayo (RIA) o ensayo de inmunoadsorción enzimática (ELISA). La afinidad de unión del anticuerpo monoclonal puede determinarse, por ejemplo, mediante el análisis Scatchard de Munson y col, *Anal. Biochem.*, 107:220 (1980).

[0320] Después de identificar que las células de hibridoma producen anticuerpos con la especificidad, afinidad o actividad deseada, o todos ellos, los clones se pueden subclonar limitando los procedimientos de dilución y pueden crecer mediante métodos estándar (Goding, *Monoclonal Antibodies: Principles and Practice*, págs. 59-103 (Academic Press, 1986)). Los medios de cultivo adecuados para este fin incluyen, por ejemplo, medio D-MEM o RPMI-1640. Además, las células de hibridoma pueden crecer *in vivo* en un animal como tumores con ascitis. Los anticuerpos monoclonales secretados por los subclones se separan adecuadamente del medio de cultivo, los fluidos ascíticos o el suero mediante procedimientos convencionales de purificación de inmunoglobulinas, como por ejemplo, la proteína-A-sefarosa, la cromatografía de hidroxilapatita, la electroforesis en gel, la diálisis o la cromatografía de afinidad.

2. Algunos métodos de detección de librerías

[0321] Los anticuerpos anti-STEAP-1 de la invención se pueden obtener utilizando bibliotecas combinatorias para detectar anticuerpos con la actividad o actividades deseadas. Por ejemplo, se conocen en la materia diversos métodos para generar bibliotecas de representación en imágenes de fagos y detectar dichas bibliotecas para anticuerpos que posean las características de unión deseadas. Estos métodos se describen de forma general en Hoogenboom y col. (2001) en *Methods in Molecular Biology* 178:1-37 (O'Brien y col., ed., Human Press, Totowa, NJ), y en ciertas formas de realización, en Lee y col. (2004) *J. Mol. Biol.* 340:1073-1093.

[0322] En principio, los clones de anticuerpos sintéticos se seleccionan mediante la detección de bibliotecas de fagos que contienen fagos que representan diversos fragmentos de la región variable (Fv) de anticuerpos fusionados a proteína de revestimiento de fagos. Estas bibliotecas de fagos se criban mediante cromatografía de afinidad para detectar el antígeno deseado. Los clones que expresan fragmentos Fv capaces de unirse al antígeno deseado son adsorbidos al antígeno y, de este modo, separados de los clones de la biblioteca que no tienen capacidad de unión. Los clones con capacidad de unión se eluyen entonces del antígeno y pueden enriquecerse aún más mediante ciclos adicionales de adsorción/elución de antígenos. Cualquiera de los anticuerpos anti-STEAP-1 de la invención se puede obtener diseñando un procedimiento apropiado de detección de antígenos para seleccionar el clon del fago de interés, seguido por la construcción de un clon del anticuerpo anti-STEAP-1 de longitud completa utilizando las secuencias de Fv del clon del fago de interés y secuencias de la región constante (Fc) adecuadas, descritas en Kabat y col., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

[0323] En determinadas formas de realización, el dominio de unión a antígeno de un anticuerpo está formado a partir de dos regiones variables (V) de unos 110 aminoácidos, una correspondiente a la cadena ligera (VL) y otra correspondiente a la cadena pesada (VH), que presentan en ambos casos tres bucles hipervariables (HVR) o regiones de determinación de complementariedad (CDR). Los dominios variables se pueden representar funcionalmente en el fago, ya sea como fragmentos de Fv de cadena única (scFv), en los que VH y VL están covalentemente enlazados por medio de un péptido corto y flexible, o como fragmentos Fab, en los que están fusionados a un dominio constante e interactúan no covalentemente, como se describe en Winter y col, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Como se usa en la presente memoria descriptiva, los clones de fagos que codifican scFv y los clones de fagos que codifican Fab se denominan colectivamente "clones de fagos Fv" o "clones Fv".

[0324] Los repertorios de genes de VH y VL se pueden clonar por separado mediante reacción en cadena de la polimerasa (PCR) y recombinar aleatoriamente en bibliotecas de fagos, en las que se puede buscar por clones de unión a antígeno, como se describe en Winter y col, *Ann. Rev. Immunol.*, 12: 433-455

(1994). Las bibliotecas procedentes de fuentes inmunizadas proporcionan anticuerpos de alta afinidad al inmunógeno sin la necesidad de construir hibridomas. Alternativamente, el repertorio virgen se puede clonar para proporcionar una única fuente de anticuerpos humanos a una gran variedad de antígenos extraños y también de autoantígenos sin ninguna inmunización como se describe en Griffiths y col, *EMBO J,* 12: 725-734 (1993). Por último, las bibliotecas nativas también se pueden producir sintéticamente mediante la clonación de segmentos de genes V no reorganizados procedentes de células madre y utilizando cebadores de la PCR que contenga secuencias aleatorias para codificar las regiones CDR3 altamente variables y para conseguir la reorganización *in vitro* como se describe en Hoogenboom y Winter, *J. Mol. Biol.*, 227: 381-388 (1992).

[0325] En determinadas formas de realización, el fago filamentoso se utiliza para representar fragmentos de anticuerpos mediante fusión a la proteína de revestimiento menor pIII. Los fragmentos de anticuerpos se pueden representar como fragmentos Fv de cadena única, en los que los dominios de VH y VL están conectados a la misma cadena polipeptídica mediante un separador de polipéptidos flexible, como se describe en Marks y col, *J. Mol. Biol.,* 222: 581-597 (1991), o como fragmentos Fab, en los que una cadena se fusiona a pIII y la otra se segregan en el periplasma del hospedero celular bacteriano donde el ensamblaje de una estructura proteínica de revestimiento de Fab se representa en la superficie del fago mediante el desplazamiento de las proteínas de revestimiento de tipo natural, por ejemplo, como se describe en Hoogenboom y col., *Nucl. Acids Res.,* 19: 4133-4137 (1991).

[0326] En general, los ácidos nucleicos que codifican fragmentos de genes de anticuerpos se obtienen a partir de inmunocitos recolectados de humanos o animales. Si se desea una biblioteca que presente un sesgo a favor de clones anti-STEAP-1, el sujeto se inmuniza con STEAP-1 para generar una respuesta a anticuerpos y las células esplénicas o los linfocitos B circulantes u otros linfocitos de la circulación periférica se recuperan para la construcción de la biblioteca. En una forma de realización preferente, una biblioteca de fragmentos de genes de anticuerpos humanos sesgada a favor de clones de anti-STEAP-1 humano se obtiene al generar una respuesta a un anticuerpo anti-STEAP-1 en ratones transgénicos portadores de una matriz de genes humanos funcionales de la inmunoglobulina (y carentes de un sistema funcional de producción de anticuerpos endógenos), de forma que la inmunización con STEAP-1 da lugar a células B productoras de anticuerpos humanos anti-STEAP-1. La generación de ratones transgénicos productores de anticuerpos humanos se describe más adelante.

[0327] El enriquecimiento adicional de poblaciones celulares reactivas a anti-STEAP-1 se puede obtener mediante un procedimiento de detección adecuado para aislar linfocitos B que expresen un anticuerpo unido a membrana específico de STEAP-1, por ejemplo, mediante separación celular con cromatografía de afinidad a STEAP-1 o adsorción de células a STEAP-1 marcada con flurocromo seguida de separación de células activadas por fluorescencia (FACS).

[0328] Alternativamente, el uso de células esplénicas y/o linfocitos B u otros PBL procedentes de un donante no inmunizado ofrece una mejor representación del posible repertorio de anticuerpos y también permite la construcción de una biblioteca de anticuerpos usando cualquier especie animal (humana o no humana) en la que STEAP-1 no es antigénico. En el caso de bibliotecas que incorporen construcción de genes de anticuerpos *in vitro*, las células madre se recolectan del sujeto para proporcionar ácidos nucleicos que codifiquen segmentos de genes de anticuerpos no organizados. Los inmunocitos de interés se pueden obtener de diversas especies animales, como humanos, ratón, rata, lagomorfa, luprina, canina, felina, porcina, bovina, equina y especies aviares, etc.

[0329] Los segmentos de regiones variables de genes anticuerpos que codifican ácidos nucleicos (incluidos los segmentos VH y VL) se recuperan a partir de las células de interés y se amplifican. En el caso de bibliotecas de genes de VH y VL reorganizadas, el ADN deseado se puede obtener aislando el ADN o el ARNm genómico de los linfocitos, seguido de la reacción en cadena de la polimerasa (PCR) con cebadores que se acoplen a los extremos 5' y 3' de los genes de la VH y la VL reorganizados, como se describe en Orlandi y col., *Proc. Natl. Acad. Sci. (USA),* 86: 3833-3837 (1989), lo que permite obtener diversos repertorios de genes V para su expresión. Los genes V se pueden amplificar a partir del ADNc y del ADN genómico con cebadores reversos en el extremo 5' del exon que codifica el dominio V maduro y cebadores anversos dentro del segmento J como se describe en Orlandi y col. (1989) y en Ward y col., *Nature*, 341: 544-546 (1989). Sin embargo, para amplificar desde el ADNc, los cebadores reversos también se pueden situar en el exón líder, como se describe en Jones y col., *Biotechnol.*, 9: 88-89 (1991), y los cebadores anversos dentro de la región constante, como se describe en Sastry y col., *Proc. Natl. Acad. Sci.* (USA), 86: 5728-5732 (1989). Para maximizar la complementariedad, se puede incorporar la degeneración en los cebadores como se describe en Orlandi y col. (1989) o Sastry y col. (1989). En ciertas formas de realización, la diversidad de bibliotecas se maximiza utilizando cebadores de la PCR dirigidos a cada familia de genes V para amplificar todas las organizaciones disponibles de VH y VL presentes en la muestra de ácidos nucleicos de inmunocitos, por ejemplo, como se describe en el método de Marks y col., *J. Mol. Biol.*, 222: 581-597 (1991) o como se describe en el método de Orum y col., *Nucleic Acids Res.*, 21: 4491-4498 (1993). Para la clonación del ADN amplificado en vectores de expresión, se pueden introducir sitios de restricción poco habituales dentro del cebador de la PCR como una marca en un extremo, según se describe en Orlandi y col. (1989), o mediante una nueva amplificación de la PCR con un cebador marcado, como se describe en Clackson y col., *Nature*, 352: 624-628 (1991).

[0330] De segmentos de genes V *in vitro* se pueden derivar repertorios de genes V sintéticamente reorganizados. La mayoría de los segmentos de genes VH humanos se han clonado y secuenciado (según Tomlinson y col. *J. Mol. Biol.*, 227: 776-798 (1992)), y mapeados (según Matsuda y col., *Nature Genet.*, 3: 88-94 (1993); estos segmentos clonados (incluidas todas las principales conformaciones del bucle H1 y H2)

se pueden utilizar para generar diversos repertorios de genes VH con cebadores de la PCR que codifican bucles de H3 de secuencia y longitud diversas, como se describe en Hoogenboom y Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Los repertorios de VH también se pueden hacer con toda la diversidad de secuencias centrada en un bucle de H3 largo de una única longitud, como se describe en Barbas y col., *Proc. Natl. Acad. Sci. USA*, 89: 4457-4461 (1992). Los segmentos Vκ y Vλ humanos se han clonado y secuenciado (según se explica en Williams y Winter, *Eur. J. Immunol.*, 23: 1456-1461 (1993)) y se pueden utilizar para hacer repertorios sintéticos de la cadena ligera. Los repertorios de genes V sintéticos, basados en una gran variedad de dominios de VH y VL, y longitudes de L3 y H3, codificarán anticuerpos de considerable diversidad estructural. Tras la amplificación de los ADN codificadores de genes V, los segmentos de genes V de línea germinal se pueden reorganizar *in vitro* según los procedimientos de Hoogenboom y Winter, *J. Mol. Biol.*, 227: 381-388 (1992).

[0331] Se pueden construir repertorios de fragmentos de anticuerpos mediante combinación de repertorios de genes de VH y VL juntos de varias formas. Cada repertorio se puede crear en vectores diferentes y los vectores se pueden recombinar *in vitro*, por ejemplo, como se describe en Hogrefe y col., *Gene*, 128: 119-126 (1993), o *in vivo* mediante infección combinatoria, por ejemplo, el sistema loxP descrito en Waterhouse y col., *Nucl. Acids Res.*, 21: 2265-2266 (1993). El procedimiento de la recombinación *in vivo* se aprovecha de las dos cadenas de los fragmentos Fab para superar el límite en el tamaño de la biblioteca impuesto por la eficacia de transformación de *E. coli*. Los repertorios de VH y VL vírgenes se clonan por separado, uno en un fagémido y el otro en un vector fágico. Las dos bibliotecas se combinan a continuación mediante infección fágica de bacterias que contienen fagémidos, de forma que cada célula contiene una combinación distinta y el tamaño de la biblioteca sólo está limitado por el número de células presente (unos 10^{12} clones). Los dos vectores contienen seales de recombinación in vivo, por lo que los genes VH y VL se recombinan en un único replicón y se introducen en viriones fágicos. Estas enormes bibliotecas proporcionan gran número de diversos anticuerpos con buena afinidad (K_d^{-1} de aproximadamente 10^{-8} M).

[0332] Alternativamente, los repertorios se pueden clonar secuencialmente en el mismo vector, por ejemplo, como se describe en Barbas y col., *Proc. Natl. Acad. Sci. USA,* 88: 7978-7982 (1991), o ensamblar juntos mediante PCR y, posteriormente, clonarse, como se describe en Clackson y col., *Nature*, 352: 624-628 (1991). El ensamblaje de la PCR también se puede utilizar para unir los ADN de la VH y la VL con ADN que codifica un espaciador peptídico flexible para formar repertorios de Fv de cadena única (scFv). En otra técnica, "el ensamblaje de la PCR en el interior de la célula" se usa para combinar genes VH y VL dentro de linfocitos mediante PCR y, posteriormente, clonar repertorios de genes enlazados, como se describe en Embleton y col., *Nucl. Acids Res.*, 20: 3831-3837 (1992).

[0333] Los anticuerpos producidos por bibliotecas nativas (ya sea naturales o sintéticas) pueden ser de afinidad moderada (K_d^{-1} de aproximadamente 10^6 a 10^7 M^{-1}), pero la maduración de la afinidad también se puede imitar *in vitro* mediante construcción y reselección a partir de bibliotecas secundarias, como se describe en Winter y col. (1994), mencionado anteriormente. Por ejemplo, la mutación se puede introducir aleatoriamente *in vitro* utilizando polimerasa con tendencia al error (explicado en Leung y col., *Technique,* 1: 11-15 (1989)) en el método de Hawkins y col., *J. Mol. Biol.,* 226: 889-896 (1992) o en el método de Gram y col., *Proc. Natl. Acad. Sci USA*, 89: 3576-3580 (1992). Asimismo, la maduración de la afinidad se puede realizar mediante mutación aleatorizada de una o más CDR, por ejemplo, utilizando PCR con cebadores de secuencia aleatoria que abarquen la CDR de interés, en clones de Fv individuales seleccionados y detecten clones de afinidad más alta. El documento WO 9607754 (publicado el 14 de marzo de 1996) describía un procedimiento para inducir la mutagenia en una región de determinación de complementariedad de una cadena ligera de inmunoglobulinas para crear una librería de genes de la cadena ligera. Otro método eficaz consiste en recombinar los dominios de VH o VL seleccionados por representación de fagos con repertorios de variantes de domino V que se producen naturalmente obtenidos a partir de donantes no inmunizados y detectar los de mayor afinidad en varias series de nueva transposición de cadenas, como se describe en Marks y col., *Biotechnol.,*10: 779-783 (1992). Esta técnica permite la producción de anticuerpos y de fragmentos de anticuerpos con afinidades del orden de 10^{-9} M o menos.

[0334] La detección de las bibliotecas se puede lograr mediante diversas técnicas conocidas en la materia. Por ejemplo, se puede utilizar STEAP-1 para revestir los pocillos de placas de adsorción, expresado en hospederos celulares en placas de adsorción o utilizada en separación celular, o conjugada con biotonina para captura con partículas revestidas de estreptavidina, o utilizadas en cualquier otro procedimiento conocido en la materia para detectar bibliotecas de representación de imágenes de fagos.

[0335] Las muestras de bibliotecas de fagos están en contacto con el STEAP-1 inmovilizado en condiciones aptas para la unión de al menos una porción de partículas de fago con el adsorbente. Normalmente, las condiciones, incluido el pH, la concentración iónica, la temperatura y similares, se seleccionan de forma que imiten condiciones fisiológicas. Los fagos unidos a la fase sólida se lavan y, posteriormente, se eluyen mediante ácido, por ejemplo, como se describe en Barbas y col., *Proc. Natl. Acad. Sci USA*, 88: 7978-7982 (1991), o mediante álcali, por ejemplo, como se describe en Marks y col., *J. Mol. Biol.,* 222: 581-597 (1991), o mediante competición antigénica con STEAP-1, por ejemplo, en un procedimiento similar al procedimiento de competición antigénica de Clackson y col., *Nature*, 352: 624-628 (1991). Los fagos se pueden enriquecer de 20 a 1.000 veces en una única serie de selección. Además, los fagos enriquecidos se pueden cultivar en cultivos bacterianos y estar sometidos a nuevas series de selección.

[0336] La eficacia de la selección depende de muchos factores, incluida la cinética de la disociación durante el lavado y si múltiples fragmentos de anticuerpos de un único fago se pueden unir simultáneamente a antígeno. Los anticuerpos con rápida cinética de disociación (y afinidades de unión débiles) se pueden conservar mediante el uso de lavados cortos, representación de imágenes de fagos multivalente y densidad de revestimiento alta del antígeno en fase sólida. La densidad elevada no sólo estabiliza al fago durante las interacciones multivalentes, sino que, además, favorece la unión del fago disociado otra vez. La selección de anticuerpos con lenta cinética de disociación (y buenas afinidades de unión) se puede promover mediante el uso de largos lavados y representación de imágenes de fagos monovalentes, como se describe en Bass y col., *Proteins*, 8: 309-314 (1990) y en el documento WO 92/09690, y una baja densidad de revestimiento del antígeno como se describe en Marks y col., *Biotechnol.*, 10: 779-783 (1992).

[0337] Es posible seleccionar entre anticuerpos de fagos de distintas afinidades, incluso con afinidades que difirieren ligeramente, para STEAP-1. Sin embargo, la mutación aleatoria de un anticuerpo seleccionado (por ejemplo, como la realizada en algunas de las técnicas de maduración de la afinidad) es probable que ocasione muchos mutantes, la mayoría de unión a antígeno, y unos cuantos con afinidad más alta. Con un STEAP-1 limitado, es raro que se pudieran completar fagos de alta afinidad. Para conservar todos los mutantes de afinidad más alta, los fagos se pueden incubar con una cantidad mayor de STEAP-1 biotinilado, pero con el STEAP-1 biotinilado a una concentración de molaridad menor de la constante de afinidad molar diana para STEAP-1. Los fagos con afinidad de unión más alta pueden, entonces, ser capturados por partículas paramagnéticas revestidas de estreptavidina. Esta "captura en equilibrio" permite seleccionar los anticuerpos según sus afinidades de unión, son una sensibilidad que permite aislar clones mutantes con una afinidad tan solo dos veces más elevada de la enorme cantidad de fagos con afinidad menor. Las condiciones usadas en el lavado de fagos unidos a una fase sólida también se pueden manipular para distinguirlos en función de la cinética de disociación.

[0338] Los clones anti-STEAP-1 se pueden seleccionar en función de la actividad. En ciertas formas de realización, la invención proporciona anticuerpos anti-STEAP-1 que se unen a células vivas que expresan naturalmente el STEAP-1. En una forma de realización, la invención proporciona anticuerpos anti-STEAP-1 que bloquean la unión entre un ligando de STEAP-1 y STEAP-1, pero no bloquean la unión entre un ligando de STEAP-1 y una segunda proteína. Los clones de Fv que corresponden a estos anticuerpos anti-STEAP-1 se pueden seleccionar (1) aislando clones anti-STEAP-1 de una biblioteca de fagos como se ha descrito antes y, opcionalmente, amplificando la población aislada de clones de fagos mediante el cultivo de la población en un hospedero bacteriano apropiado; (2) seleccionando STEAP-1 y una segunda proteína contra la que se desea una actividad bloqueante y no bloqueante, respectivamente; (3) adsorbiendo los clones de fagos anti-STEAP-1 a STEAP-1 inmovilizado; (4) usando una mayor cantidad de

la segunda proteína para eluir los clones no deseados que reconocen los determinantes de unión a STEAP-1 que se solapan o se comparten con los determinantes de unión de la segunda proteína y (5) elución de los clones que todavía no se han adsorbido tras el paso (4). Opcionalmente, los clones con las propiedades bloqueantes/no bloqueantes deseadas se pueden enriquecer también mediante repetición de los procedimientos de selección descritos en la presente memoria descriptiva una o más veces.

[0339] El ADN que codifica los anticuerpos monoclonales derivados de hibridroma o los clones de Fv de la invención es rápidamente aislado y secuenciado utilizando procedimientos convencionales (por ejemplo, utilizando cebadores oligonucleóticos diseñados para amplificar específicamente las regiones que codifican las cadenas ligera y pesada de interés de plantillas de ADN de fagos o hibridoma). Una vez aislado, el ADN se puede colocar en vectores de expresión, que después se transfectan en células anfitrión, como por ejemplo las células E. coli, las células COS de monos, las células de ovario de hámster chino (CHO) o las células de mieloma que, de otro modo, no producen la proteína de la inmunoglobulina, para obtener la síntesis de los anticuerpos monoclonales deseados en las células anfitrión recombinantes. Los artículos de revisión sobre expresión recombinante en bacterias del ADN que codifican el anticuerpo incluyen Skerra y col., *Curr. Opinion in Immunol.*, 5: 256 (1993) y Pluckthun, *Immunol. Revs*, 130: 151 (1992).

[0340] El ADN que codifica los clones de Fv de la invención se pueden combinar con secuencias de ADN conocidas que codifican las regiones constantes de la cadena ligera y/o la cadena pesada (por ejemplo, las secuencias de ADN apropiadas se pueden obtener según Kabat y col., mencionado anteriormente) para formar clones que codifiquen cadenas ligeras y/o pesadas de longitud total o parcial. Se tendrá en cuenta que las regiones constantes de cualquier isótopo se pueden utilizar para este fin, incluidas las regiones constantes IgG, IgM, IgA, IgD e IgE, y que dichas regiones constantes se pueden obtener de cualquier especie animal o humana. Un clon de Fv se deriva del ADN del dominio variable de una especie animal (por ejemplo, un humano) y, luego, se fusiona al ADN de la región constante de otra especie animal para formar una o más secuencias de codificación, puesto que una cadena ligera y/o pesada de longitud completa e "híbrida" está incluida en la definición de anticuerpo "híbrido" y "quimérico" como se usa en la presente memoria descriptiva. En determinadas formas de realización, un clon de Fv derivado de ADN variable humano se fusiona con ADN de región constante humano para formar una o más secuencias de codificación para todas las cadenas ligeras y/o pesadas de longitud parcial o completa y humanas.

[0341] El ADN que codifica un anticuerpo anti-STEAP-1 derivado de un hibridoma de la invención también se puede modificar, por ejemplo, mediante sustitución de las secuencias murinas homólogas derivadas del clon de hibridoma por la secuencia de codificación para los dominios constantes de la cadena ligera y pesada humanas (por ejemplo, como en el método de Morrison y col., *Proc. Natl.*

Acad. Sci. USA, 81: 6851-6855 (1984)). El ADN que codifica un anticuerpo o fragmento derivado del clon de Fv o un hibridoma se puede modificar a su vez mediante el enlace covalente a la secuencia de codificación de la inmunoglobulina de la totalidad o parte de la secuencia de codificación de un polipéptido que no sea inmunoglobulina. De este modo, se preparan anticuerpos "quiméricos" o "híbridos" que tiene la especificidad de unión de los anticuerpos de la invención derivados del clon de hibridoma o del clon de Fv.

3. Vectores, células hospederas y métodos recombinantes

[0342] Para la producción recombinante de un anticuerpo de la invención, el ácido nucleico que lo codifica se aísla e inserta en un vector replicable para la clonación subsiguiente (amplificación del ADN) o para expresión. El ADN que codifica el anticuerpo se aísla fácilmente y se secuencia utilizando procedimientos convencionales (por ejemplo, usando sondas oligonucleótidas capaces de unirse específicamente a los genes que codifican las cadenas pesadas y ligeras del anticuerpo). Hay muchos vectores disponibles. La elección del vector depende en parte del hospedero celular que se utilice. Por lo general, las células hospederas son de origen procariótico o eucariótico (por lo general, de mamíferos). Se tendrá en cuenta que las regiones constantes de cualquier isótopo se pueden utilizar para este fin, incluidas las regiones constantes IgG, IgM, IgA, IgD e IgE, y que dichas regiones constantes se pueden obtener de cualquier especie animal o humana.

Generación de anticuerpos usando células hospederas eucarióticas:

Construcción de vectores

[0343] Las secuencias de polinucleótidos que codifican los componentes de polipéptidos del anticuerpo de la invención se pueden obtener utilizando técnicas recombinantes estándar. Las secuencias deseadas de polinucleótidos se pueden aislar y secuenciar a partir de anticuerpos que producen células, como por ejemplo células de hibridoma. Alternativamente, los polinucleótidos se pueden sintetizar usando técnicas de PCR o sintetizador de nucleótidos. Una vez obtenidas, las secuencias que codifican los polipéptidos se insertan en un vector recombinante capaz de replicar y expresar polinucleótidos heterólogos en los hospederos procarióticos. Muchos vectores que están disponibles y son conocidos en la materia se pueden utilizar para el fin de la presente invención. La selección de un vector apropiado dependerá principalmente del tamaño de los ácidos nucleicos que se van a insertar en el vector y de la célula hospedera concreta que se transformará con el vector. Cada vector contiene diversos componentes, dependiendo de su función (amplificación o expresión de polinucleótido heterólogo o ambas) y su compatibilidad con la célula hospedera concreta en la que resida. Por lo general, los componentes del vector incluyen, entre otros: un origen de replicación, un gen marcador de la selección, un promotor, un sitio de unión a ribosoma (RBS), una secuencia de señal, la inserción del ácido nucleico heterólogo y una secuencia de terminación de la trascripción.

[0344] En general, los vectores plásmidos que contienen secuencias de control y replicón que se derivan de especies compatibles con la célula hospedera se usan en conexión con estos hospederoses. Por lo general, el vector lleva un sitio de replicación, así como secuencias de marcado, que son capaces de proporcionar selección fenotípica en células transformadas. Por ejemplo, *E. coli* se transforma habitualmente usando pBR322, un plásmido derivado de una especie *E. coli*. El pBR322 contiene genes que codifican la resistencia a la ampicilina (Amp) y tetraciclina (Tet) y, de este modo, proporcionan una forma fácil de identificar células transformadas. El pBR322, sus derivados, u otros plásmidos microbianos o bacteriófagos pueden contener también, o ser modificados para que contengan, promotores que pueden ser utilizados por el organismo microbiano para la expresión de proteínas endógenas. Ejemplos de derivados del pBR322 usados para la expresión de anticuerpos concretos se describen en detalle en Carter y col., patente de EE.UU. N.º 5.648.237.

[0345] Además, los vectores fágicos que contienen secuencias de control y replicón que son compatibles con los microorganismos hospederos se pueden usar como vectores de transformación en relación con estos hospederos. Por ejemplo, un bacteriófago como λGEM.TM.-11 se puede utilizar para marcar un vector recombinante que se pueda utilizar para transformar células hospederas susceptibles como *E. coli* LE392.

[0346] El vector de expresión de la invención puede comprender dos o más pares de promotores de cistrón, que codifiquen cada uno de los componentes polipeptídicos. Un promotor es una secuencia reguladora no traducida situada antes del extremo (5') del promotor en un cistrón que modula su expresión. Los promotores procarióticos suelen pertenecer a dos clases: inducibles y constitutivos. El promotor inducible es aquel que inicia un aumento de los niveles de trascripción del cistrón bajo su control en respuesta a cambios en las condiciones del cultivo, por ejemplo, la presencia o ausencia de un nutriente o un cambio de temperatura.

[0347] Se conoce un gran número de promotores reconocidos por diversas células hospederas posibles. El promotor seleccionado puede enlazarse funcionalmente al ADN del cistrón que codifica la cadena pesada o ligera mediante eliminación del promotor del ADN fuente a través de digestión enzimática mediante restricción e inserción de la secuencia aislada del promotor en el vector de la invención. Tanto la secuencia nativa del promotor como muchos promotores heterólogos se pueden utilizar para dirigir la amplificación y/o expresión de los genes diana. En algunas formas de realización, se utilizan promotores heterólogos, puesto que por lo general permiten una mayor transcripción y una mayor producción del gen diana expresado, frente al promotor polipeptídico diana nativo.

[0348] Los promotores aptos para su uso con hospederos procarióticos incluyen el promotor PhoA, los sistemas promotores de lactosa y β-galactamasa, un sistema promotor a base de triptofano (trp) y promotores híbridos como el promotor tac o el trc. Sin embargo, también son aptos otros promotores que

son funcionales en bacterias (como otros promotores de fagos o bacterianos conocidos). Se han publicado sus secuencias de nucleótidos, lo que permite que un experto pueda ligarlas a cistrones que codifiquen las cadenas pesada y ligera dianas (Siebenlist y col., (1980) *Cell* 20: 269) usando enlazadores o adaptadores que proporcionen sitios de restricción necesarios.

[0349] En un aspecto de la invención, cada cistrón dentro del vector recombinante comprende un componente de la secuencia de señal de la secreción que dirige la translocación de los polipéptidos expresados en una membrana. En general, la secuencia de señal puede ser un componente del vector, o puede ser parte del ADN del polipéptido diana que se inserta en el vector. La secuencia de señal seleccionada a efectos de esta invención es aquella que la célula hospedera reconoce y procesa (es decir, escindida por una peptidasa de señal). Para las células hospederas procarióticas que no reconocen ni procesan las secuencias de señal nativas a los polipéptidos heterólogos, la secuencia de señal se sustituye por una secuencia de señal procariótica seleccionada, por ejemplo, entre un grupo formado por fosfatasa alcalina, penicilasa, lpp o líderes de enterotoxina II termoestable (STII), LamB, PhoE, PelB, OmpA y MBP. En una forma de realización de la invención, las secuencias de señal usadas en ambos cistrones del sistema de expresión son secuencias de señal STII o variantes de las mismas.

[0350] En otro aspecto, la producción de las inmunoglobulinas según la invención puede tener lugar en el citoplasma de la célula hospedera y, por lo tanto, no requiere la presencia de secuencias de señal de secreción dentro de cada cistrón. A este respecto, las cadenas pesada y ligera de inmunoglobulina se expresan, multiplican y ensamblan para formar inmunoglobulinas funcionales dentro del citoplasma. Determinadas cepas de hospederos (por ejemplo, las cepas de *E. coli* trxB) ofrecen condiciones citoplasmáticas que son favorables a la formación de uniones de disulfuros, lo que permite una multiplicación y ensamblaje correctos de las subunidades de las proteínas expresadas. Proba y Pluckthun *Gene*, 159:203 (1995).

[0351] Los anticuerpos de la invención también se pueden producir usando un sistema de expresión en el que la relación cuantitativa de los componentes polipeptídicos expresados se pueda modular para maximizar la producción de anticuerpos secretados y debidamente ensamblados de la invención. Esta modulación se logra al menos en parte mediante modulación simultánea de concentraciones translacionales para los componentes polipeptídicos.

[0352] Una técnica para la modulación de la concentración translacional se divulga en Simmons y col., patente de EE.UU. N.º 5.840.523. Utiliza variantes de la región de iniciación translacional (TIR) dentro de un cistrón. Para una TIR dada, se puede crear una serie de variantes de secuencias de ácidos nucleicos o aminoácidos con un intervalo de concentraciones translacionales, lo que proporciona un medio conveniente mediante el cual ajustar este factor al nivel de expresión deseado de la cadena específica. Las variantes de la TIR se pueden generar mediante técnicas de mutagenia convencionales que

dan lugar a cambios en codones que pueden alterar la secuencia aminoacídica. En ciertas formas de realización, los cambios en la secuencia de nucleótidos son silenciosos. Las alteraciones en la TIR pueden incluir, por ejemplo, alteraciones en el número o espaciado de secuencias de Shine-Dalgarno, junto con alteraciones en la secuencia de la señal. Un método para generar secuencias de señal mutantes es generar un "banco de codones" al inicio de una secuencia de codificación que no cambie la secuencia de aminoácidos de la secuencia de la señal (es decir, los cambios son silenciosos). Esto se puede conseguir cambiando la posición del tercer nucleótido de cada codón; además, algunos aminoácidos, como leucina, serina y arginina, tienen múltiples posiciones primera y segunda que pueden añadir complejidad para realizar el banco. Este método de mutagenia se describe en detalle en Yansura y col., (1992) *METHODS: A Companion to Methods in Enzymol.* 4:151-158.

[0353] En una forma de realización, un conjunto de vectores se genera con un intervalo de concentraciones de TIR para cada cistrón en ellos. Este conjunto limitado permite una comparación de los niveles de expresión de cada cadena, así como la producción de los productos deseados de anticuerpos en varias combinaciones de concentraciones de TIR. Las concentraciones TIR se pueden determinar cuantificando el nivel de expresión de un gen indicador como se describe en detalle en Simmons y col., patente de EE.UU. N.° 5.840.523. En función de la comparación de las concentraciones translacionales, las TIR individuales se seleccionan para su combinación en los constructos del vector de expresión de la invención.

[0354] Las células hospederas procarióticas aptas para la expresión de anticuerpos de la invención incluyen Archaebacteria y Eubacteria, como microorganismos grampositivos o gramnegativos. Entre los ejemplos de bacterias útiles se incluyen Escherichia (por ejemplo, *E. coli*), Bacilli (por ejemplo, B. subtilis), Enterobacteria, las especies Pseudomonas (por ejemplo, P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla o Paracoccus. En una forma de realización, se utilizan células gramnegativas. En una forma de realización, se utilizan células *E. coli* como hospederas para la invención. Entre los ejemplos de cepas *E. coli* se incluyen la cepa W3110 (Bachmann, *Cellular and Molecular Biology*, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), págs. 1190-1219; depósito en la ATCC N.° 27.325) y derivados de las mismas, que incluyen la cepa 33D3 con el genotipo W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR (patente de EE.UU. N.° 5.639.635). Otras cepas y derivados de las mismas, como *E. coli* 294 (ATCC 31.446), *E. coli* B, E. coliλ 1776 (ATCC 31.537) y *E. coli* RV308 (ATCC 31.608) también están indicados. Estos ejemplos tienen carácter ilustrativo y no limitador. Los métodos para la construcción de derivados de cualquiera de las bacterias antes mencionadas con genotipos definidos son conocidos en la materia y se describen, por ejemplo, en Bass y col., *Proteins*, 8:309-314 (1990). Por lo general, es necesario seleccionar las bacterias apropiadas teniendo en cuenta la replicabilidad del replicón en las

células de una bacteria. Por ejemplo, las especies *E. coli*, Serratia o Salmonella se pueden usar bien como hospederos cuando plásmidos bien conocidos como pBR322, pBR325, pACYC177 o pKN410 se utilizan para suministrar al replicón. Normalmente, en caso de que la célula hospedera deba secretar cantidades mínimas de enzimas proteolíticas puede resultar conveniente incorporar al cultivo celular inhibidores adicionales de la proteasa.

Producción de anticuerpos

[0355] Las células hospederas se transforman con los vectores de expresión descritos anteriormente y se cultivan en medios nutrientes convencionales modificados como es adecuado para inducir promotores, seleccionar transformantes o amplificar los genes que codifican las secuencias deseadas.

[0356] Transformación significa introducir ADN en el hospedero procariótico de forma que el ADN sea replicable, ya sea como elemento extracromosómico o como integrante cromosómico. Según la célula hospedera que se utilice, la transformación se realiza empleando técnicas estándar apropiadas para dichas células. El tratamiento con calcio que emplea cloruro de calcio se usa por lo general para células bacterianas que contienen barreras sustanciales en la pared celular. Otro método de transformación emplea polietilenglicol/DMSO. Otra técnica utilizada es la electroporación.

[0357] Las células procarióticas utilizadas para producir los polipéptidos de la invención se cultivan en medios conocidos en la materia y aptos para el cultivo de células hospederas seleccionadas. Ejemplos de medios apropiados son caldo de luria (LB) más nutrientes complementarios necesarios. En algunas formas de realización, los medios también contienen un agente de selección, elegido en función de la construcción del vector de expresión, para permitir el crecimiento selectivo de células procarióticas que contengan el vector de expresión. Por ejemplo, se añade ampicilina a los medios de cultivo para el crecimiento de células que expresan un gen resistente a ampicilina.

[0358] Los anticuerpos necesarios aparte de carbono, nitrógeno y fuentes inorgánicas de fosfato también se pueden incluir a concentraciones apropiadas introducidas solas en una mezcla con otro suplemento o medio de cultivo como una fuente de nitrógeno compleja. Opcionalmente, el medio de cultivo puede contener uno o más agentes reductores seleccionados a partir del grupo formado por glutationa, cisteína, cistamina, tioglicolato, ditioeritriol y ditiotreitol.

[0359] Las células hospederas procarióticas se cultivan a temperaturas apropiadas. En algunas formas de realización, para el crecimiento de *E. coli*, las temperaturas oscilan entre aproximadamente 20 °C y aproximadamente 39 °C; entre aproximadamente 25 °C y aproximadamente 37 °C; o aproximadamente 30 °C. El pH del medio de cultivo puede ser cualquiera comprendido entre 5 y 9,

dependiendo principalmente del organismo hospedero. En ciertas formas de realización, el pH para *E. coli* puede oscilar entre 6,8 y 7,4 aproximadamente o 7,0 aproximadamente.

[0360] Si un promotor inducible se utiliza en el vector de expresión de la invención, la expresión de la proteína se induce en condiciones aptas para la activación del promotor. En un aspecto de la invención, los promotores PhoA se utilizan para controlar la transcripción de los polipéptidos. Según esto, las células hospederas transformadas se cultivan en un medio limitador de fosfato para la inducción. En algunas formas de realización, el medio de cultivo limitador de fosfato es el C.R.A.P (véase, por ejemplo, Simmons y col., *J. Immunol. Methods* (2002), 263:133-147). Se pueden utilizar otros inductores diversos, según el constructo de vector empleado, como se sabe en la materia.

[0361] En una forma de realización, los polipéptidos expresados de la presente invención se secretan en el periplasma de las células hospederas y se recuperan allí. La recuperación de las proteínas generalmente supone la ruptura de los microorganismos, por lo general mediante choque osmótico, sonicación o lisis. Una vez producida la ruptura de las células, los desechos celulares o células enteras se pueden eliminar mediante centrifugación o filtración. Las proteínas pueden purificarse más, por ejemplo, mediante cromatografía de afinidad con resina. De manera alternativa, pueden transportarse proteínas dentro de los medios de cultivo y aislarse en él. Las células pueden retirarse del cultivo, y el sobrenadante del cultivo puede filtrarse y concentrarse para proceder a la posterior purificación de las proteínas producidas. Los polipéptidos expresados pueden volver a aislarse e identificarse mediante métodos comúnmente conocidos, como la electroforesis en geles de poliacrilamida (PAGE) y ensayo Western blot.

[0362] En uno de los aspectos de la invención, la producción de anticuerpos se realiza en gran cantidad mediante proceso de fermentación. Existen varios procesos disponibles de fermentación de lotes alimentados a gran escala para la producción de proteínas recombinantes. Las fermentaciones a gran escala se realizan en dispositivos de al menos 1.000 litros de capacidad y, en ciertas formas de realización, entre 1.000 y 100.000 litros de capacidad. Estos fermentadores utilizan agitadores de hélice para distribuir oxígeno y nutrientes, especialmente glucosa (la fuente preferente de carbono/energía). La fermentación a pequeña escala hace referencia generalmente a la fermentación en fermentadores de aproximadamente no más de 100 litros de capacidad volumétrica, y pueden variar de 1 a 100 litros aproximadamente.

[0363] En un proceso de fermentación, la inducción de expresión proteica se inicia típicamente después de que las células hayan crecido en condiciones adecuadas hasta alcanzar la densidad deseada, por ejemplo una OD_{550} de entre 180-220, momento en el cual las células se encuentran en la fase estacionaria temprana. Pueden usarse distintos inductores, de acuerdo con el constructo del vector utilizado, tal y como se conoce en la materia y como se ha descrito anteriormente. Las células pueden hacerse crecer durante períodos más cortos antes de la inducción. Las células se inducen normalmente durante unas 12-50 horas, aunque es posible utilizar tiempos de inducción más prolongados o más cortos.

[0364] Pueden modificarse distintas condiciones de fermentación para mejorar el rendimiento de producción y la calidad de los polipéptidos de la invención. Por ejemplo, para mejorar el ensamblaje adecuado y el plegamiento de los polipéptidos de anticuerpos secretados, pueden utilizarse vectores adicionales que sobreexpresan la proteína chaperona, tales como proteínas Dsb (DsbA, DsbB, DsbC, DsbD y/o DsbG) o FkpA (una peptidil-propil cis,trans-isomerasa con actividad chaperona) para co-transformar las células hospederas procarióticas. Se ha demostrado que las proteínas chaperonas facilitan la solubilidad y el plegamiento adecuado de las proteínas heterólogas producidas en células bacterianas hospederas. Chen y col., (1999) *J Bio Chem* 274:19601-19605; Georgiou y col., patente de EE.UU. N.º 6.083.715; Georgiou y col., patente de EE.UU. N.º 6.027.888; Bothmann y Pluckthun (2000) *J. Biol. Chem.* 275:17100-17105; Ramm y Pluckthun (2000) *J. Biol. Chem.* 275:17106-17113; Arie y col., (2001) *Mol. Microbiol.* 39:199-210.

[0365] Pueden utilizarse ciertas cepas hospederas con deficiencia de enzimas proteolíticas para minimizar la proteólisis de proteínas heterólogas (especialmente las que son sensibles desde el punto de vista proteolítico). Por ejemplo, las cepas de células hospederas pueden modificarse para efectuar mutaciones genéticas relativas a los genes que codifican proteasas bacterianas conocidas, como la Proteasa III, OmpT, DegP, Tsp, Proteasa I, Proteasa Mi, Proteasa V, Proteasa VI y combinaciones de éstas. Algunas cepas de *E. coli* con deficiencia de proteasas están disponibles, y descritas, por ejemplo en Joly y col. (1998), citado anteriormente; Georgiou y col., patente de EE.UU. N.º 5.264.365; Georgiou y col., patente de EE.UU. N.º 5.508.192; Hara y col., *Microbial Drug Resistance*, 2:63-72 (1996).

[0366] En una forma de realización, en el sistema de expresión de la invención se utilizan como células hospederas cepas de *E. coli* con deficiencia de enzimas proteolíticas transformadas con plásmidos que sobreexpresan una o más proteínas chaperonas.

Purificación de anticuerpos

[0367] En una forma de realización, la proteína de anticuerpo que se produce mediante la invención descrita en la presente memoria se purifica más para obtener preparaciones que son substancialmente homogéneas para posteriores ensayos y usos. Pueden emplearse métodos estándar conocidos en la materia para la purificación de proteínas. Los siguientes procedimientos tienen carácter ejemplar en lo que se refiere a procedimientos de purificación adecuados: fraccionamiento en columnas de intercambio iónico o de inmunoafinidad, precipitación en etanol, HPLC de fase reversa, cromatografía en anhídrido silícico o en una resina de intercambio catiónico tal como DEAE, cromatoisoenfoque, SDS-PAGE, precipitación en sulfato de amonio y filtrado en gel mediante, por ejemplo, Sephadex G-75.

[0368] En un aspecto, se utiliza Proteína A inmovilizada en una fase sólida para purificación por inmunoafinidad de los productos de anticuerpos de la invención. La proteína A es una proteína de pared celular de 41kD del *Staphylococcus aureas* que se une con gran afinidad por la región Fc de los

anticuerpos. Lindmark y col., (1983) *J. Immunol. Meth.* 62:1-13. La fase sólida a la que se fija la Proteína A puede ser una columna que contenga una superficie de vidrio o anhídrido silícico, o una columna de ácido silícico o de vidrio poroso controlado. En ciertas aplicaciones, la columna se recubre con un reactivo, como el glicerol, para evitar una posible adherencia inespecífica de contaminantes.

[0369] Como primer paso de purificación, una preparación derivada del cultivo celular, según lo descrito anteriormente, puede aplicarse a una fase sólida de Proteína A inmovilizada para permitir la unión específica del anticuerpo de interés a la Proteína A. La fase sólida se lavaría a continuación, para eliminar los contaminantes unidos inespecíficamente a ella. Finalmente, el anticuerpo de interés se recupera de la fase sólida mediante elución.

Generación de anticuerpos usando células hospederas eucarióticas:

[0370] Un vector para utilizar en una célula hospedera eucariótica incluye, por lo general, uno o más de los siguientes componentes no limitantes: una secuencia de señal, un origen de replicación, uno o más genes marcadores, un potenciador, un promotor y una secuencia de finalización de la transcripción.

Componente de secuencia de señal

[0371] Un vector para utilizar en una célula hospedera eucariótica puede contener también una secuencia de señal u otro polipéptido con una escisión específica en el N-terminal de la proteína madura o polipéptido de interés. La secuencia de señal heteróloga seleccionada puede ser aquella que la célula hospedera reconoce y procesa (es decir, escindida por una peptidasa de señal). En la expresión celular de los mamíferos, están disponibles las secuencias de señal de los mamíferos así como los líderes secretores víricos, por ejemplo, la señal del herpes simplex gD. El ADN para la región de dicho precursor está ligado en la estructura de lectura al ADN que codifica el anticuerpo.

Origen de la replicación

[0372] Por lo general, no es necesario un componente origen de la replicación para los vectores de expresión en mamíferos. Por ejemplo, el origen SV40 se puede utilizar habitualmente sólo porque contiene el promotor precoz.

Componente gen de selección

[0373] Los vectores de expresión y clonación pueden contener un gen de selección, también denominado marcador seleccionable. Los genes de selección típicos codifican proteínas que (a) otorgan resistencia a antibióticos u otras toxinas, por ejemplo, ampicilina, neomicina, metotrexato o tetraciclina, (b) complementan las deficiencias auxotróficas, si procede o (c) suministran nutrientes básicos no disponibles a partir de medios complejos.

[0374] Un ejemplo de un esquema de selección utiliza un fármaco para detener el crecimiento de una célula hospedera. Estas células que se transforman con éxito con un gen heterólogo producen una proteína que otorga resistencia al fármaco y, así, sobrevive a la estrategia de selección. Ejemplos de esta selección dominante utilizan los fármacos neomicina, ácido micofenólico e higromicina.

[0375] Otro ejemplo de marcadores seleccionables adecuados para células de mamíferos son los que permiten la identificación de las células encargadas de absorber el ácido nucleico del anticuerpo, como DHFR, timidina cinasa, metalotioneína I y II, preferiblemente, genes de metalotioneína en primates, adenosina deaminasa, ornitina decarboxilasa, etc.

[0376] Por ejemplo, en algunas formas de realización, las células transformadas con el gen de selección DHFR se identifican primero a través del cultivo de todos los transformantes en un medio de cultivo que contiene metotrexato (Mtx), un antagonista competitivo del DHFR. En algunas formas de realización, una célula hospedera adecuada cuando se utiliza el DHFR de tipo natural es la línea celular de ovario de hámster chino (CHO) deficiente en actividad del DHFR (por ejemplo, ATCC CRL-9096).

[0377] Alternativamente, las células hospederas (en especial los hospederos naturales que contienen DHFR endógeno) transformadas o co-transformadas con secuencias de ADN que codifican un anticuerpo, la proteína DHFR natural y otro marcador seleccionable como la aminoglicosida 3'-fosfotransferasa (APH) puede seleccionarse mediante crecimiento celular en un medio que contenga un agente de selección para el marcador seleccionable, como un antibiótico aminoglicosídico, por ejemplo, canamicina, neomicina o G418. Véase la patente de EE.UU. N.º 4.965.199.

Componente promotor

[0378] Los vectores de expresión y clonación suelen contener un promotor que es reconocido por el organismo hospedero y está operativamente enlazado al ácido nucleico que codifica un polipéptido de interés (por ejemplo, un anticuerpo). Se conocen las secuencias de promotores para eucariotas. Por ejemplo, prácticamente todos los genes eucarióticos poseen una región rica en AT situada aproximadamente entre las bases 25 y 30 antes del extremo 5' del promotor desde donde se inicia la transcripción. Otra secuencia encontrada entre las bases 70 y 80 antes del extremo 5' del promotor desde el inicio de la transcripción de muchos genes es la región CNCAAT en la que N puede ser cualquier nucleótido. En el extremo 3' de la mayoría de los genes eucarióticos se encuentra una secuencia AATAAA que puede ser la señal para la adición de la cola de poli(A) al extremo 3' de la secuencia de codificación. En ciertas formas de realización, la totalidad o algunas de estas secuencias se pueden insertar sin problemas en vectores de expresión eucarióticos.

[0379] La trascripción a partir de los vectores en células hospederas de mamíferos está controlada, por ejemplo, por promotores obtenidos a partir de los genomas de virus como el poliomavirus,

el virus de la viruela de las aves de corral, el adenovirus (como el Adenovirus 2), el virus del papiloma bovino, el virus del sarcoma aviario, el citomegalovirus, un retrovirus, el virus de la hepatitis B y el Simian Virus 40 (SV40); a partir de promotores heterólogos de mamíferos, por ejemplo, el promotor de actina o un promotor de inmunoglobulina, o a partir de promotores de choque térmico, siempre que dichos promotores sean compatibles con los sistemas de las células hospederas.

[0380] Los promotores tempranos y tardíos del virus SV40 se obtienen fácilmente como un fragmento de restricción del SV40, que también contiene el origen viral de replicación del SV40. El promotor temprano inmediato del citomegalovirus humano se obtiene fácilmente como un fragmento de restricción HindIII E. Un sistema para la expresión del ADN en hospederos de mamíferos que utilizan el virus del papiloma bovino como vector se da a conocer en la patente de EE.UU N.º 4.419.446. Una modificación de este sistema se describe en la patente de EE.UU N.º 4.601.978. Véase también Reyes y col., *Nature* 297:598-601 (1982) para la expresión del ADNc del interferón beta humano en células de ratón bajo el control de un promotor timidina cinasa del virus del herpes simplex. Alternativamente, la repetición del terminal largo del virus del sarcoma de Rous se puede utilizar como promotor.

Componente elemento potenciador

[0381] La trascripción de ADN que codifica un anticuerpo de esta invención a través de eucariotas más altas se aumenta con frecuencia al insertar la secuencia de un potenciador en el vector. Se conocen ya muchas secuencias de potenciadores de genes de mamíferos (globina, elastasa, albúmina, fetoproteína alfa e insulina). No obstante, lo habitual es que se utilice un potenciador procedente de un virus de células eucarióticas. Entre los ejemplos destacan el potenciador SV40 en el lado tardío del origen de la replicación (bp 100-270), el potenciador del promotor temprano de citomegalovirus, el potenciador de polioma en el lado tardío del origen de la replicación y los potenciadores de adenovirus. Véase también Yaniv, *Nature* 297:17-18 (1982), que describe los elementos de potenciación para activar los promotores eucarióticos. El potenciador puede empalmarse en el vector en una posición 5' a 3' a la secuencia de codificación del polipéptido del anticuerpo, pero está situado generalmente en un sitio 5' desde el promotor.

Componente de finalización de la transcripción

[0382] Los vectores de expresión utilizados en células hospederas eucarióticas pueden también contener secuencias necesarias para la terminación de la transcripción y para la estabilización del ARNm. Estas secuencias están habitualmente disponibles desde las regiones no traducidas del extremo 5' y ocasionalmente el 3' de los ADN o ADNc virales o eucarióticos. Estas regiones contienen segmentos de nucleótidos transcritos como fragmentos poliadenilados en la porción no traducida del ARNm que codifica un anticuerpo. Un componente de finalización de la transcripción útil es la región de poliadenilación de la hormona de crecimiento bovina. Véase el documento WO94/11026 y el vector de expresión que se da a conocer en el mismo.

Selección y transformación de células hospederas

[0383] Entre las células hospederas apropiadas para clonación o expresión del ADN en los vectores de la presente memoria descriptiva figuran las células eucariotas más altas, incluidas las células hospederas de vertebrados. La propagación de células de vertebrados en cultivos (cultivo tisular) se ha convertido en un procedimiento habitual. Ejemplos de líneas celulares útiles de mamíferos son la línea CV1 de riñón del mono transformada por SV40 (COS-7, ATCC CRL 1651); la línea de riñón embrionario humano (293 ó 293 células subclonadas para crecimiento en cultivo en suspensión, Graham y col., J. *Gen Virol.* 36:59 (1977)) ; células de riñón de crías de hámster (BHK, ATCC CCL 10); células de ovario de hámster chino/-DHFR (CHO, Urlaub y col., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); células sertoli de ratón (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); células de riñón de mono (CV1 ATCC CCL 70); células de riñón de mono verde africano (VERO-76, ATCC CRL-1587); células de carcinoma cervical humano (HELA, ATCC CCL 2); células caninas de riñón (MDCK, ATCC CCL 34); células de riñón de ratas búfalo (BRL 3A, ATCC CRL 1442); células humanas de pulmón (W138, ATCC CCL 75); células hepáticas humanas (Hep G2, HB 8065); tumor de mama de ratón (MMT 060562, ATCC CCL51); células TRI (Mather y col., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); células MRC 5; células FS4 y una línea de hepatoma humano (Hep G2).

[0384] Las células hospederas se transforman con los vectores de expresión o clonación descritos anteriormente para la producción del anticuerpo y se cultivan en medios nutrientes convencionales modificados como es adecuado para inducir promotores, seleccionar transformantes o amplificar los genes que codifican las secuencias deseadas.

Cultivo de células hospederas

[0385] Las células hospederas utilizadas para producir un anticuerpo de esta invención se pueden cultivar en diversos medios. Medios comercialmente disponibles como Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma) y Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) son aptos para el cultivo de células hospederas. Además, cualquiera de los medios de cultivo descritos en Ham y col., *Meth. Enz.* 58:44 (1979), Barnes y col., *Anal. Biochem.* 102:255 (1980), patentes de EE.UU. N.º 4.767.704, 4.657.866, 4.927.762, 4.560.655 ó 5.122.469; el documento WO 90/03430; el documento WO 87/00195; o la patente de EE.UU. Ref. 30.985 se pueden utilizar como medios de cultivo para células hospederas. Cualquiera de estos medios se puede complementar cuando sea necesario con hormonas y otros factores de crecimiento (como insulina, transferrina o factor de crecimiento epidérmico), sales (como cloruro sódico, calcio, magnesio y fosfato), tampones (como HEPES), nucleótidos (como adenosina y timidina), antibióticos (como el fármaco GENTAMYCIN™), elementos de traza (definidos como compuestos inorgánicos presentes por lo habitual en las concentraciones finales en el rango micromolar) y glucosa o una fuente de energía equivalente. También pueden incluirse otros

suplementos en las concentraciones adecuadas conocidas por los expertos en la materia. Las condiciones del cultivo, tales como la temperatura o el pH, entre otras, son las utilizadas anteriormente con la célula hospedera seleccionada para la expresión y serán evidentes para los expertos.

Purificación del anticuerpo

[0386] Al utilizar técnicas recombinantes, se puede producir el anticuerpo intracelularmente o directamente secretado en el medio. Si el anticuerpo se produce intracelularmente, como primer paso, los residuos de partículas, ya sean células hospederas o fragmentos lisados, se pueden eliminar, por ejemplo, mediante centrifugación o ultrafiltrado. Cuando el anticuerpo es secretado en el medio, los sobrenadantes de dichos sistemas de expresión se pueden concentrar primero utilizando un filtro para concentración de proteínas disponible comercialmente, por ejemplo una unidad de ultrafiltrado Amicon o Millipore Pellicon. Se puede incluir un inhibidor de la proteasa como PMSF en cualquiera de las fases anteriores para inhibir la proteólisis y se pueden incluir antibióticos para impedir el crecimiento de contaminantes casuales.

[0387] La composición del anticuerpo preparado a partir de las células se puede purificar utilizando, por ejemplo, cromatografía de hidroxilapatita, electroforesis en gel, diálisis y cromatografía de afinidad. Esta última es una técnica de purificación conveniente. La idoneidad de la proteína A como ligando de afinidad depende de las especies y del isotipo de cualquier domino Fc de la inmunoglobulina que está presente en el anticuerpo. La proteína A se puede utilizar para purificar anticuerpos que se basan en cadenas pesadas humanas γ1, γ2, o γ4 (Lindmark y col., J. *Immunol. Methods* 62:1-13 (1983)). La proteína G se recomienda para todos los isotipos de ratón y para la γ3 humana (Guss y col., EMBO J. 5:15671575 (1986)). La matriz a la que el ligando de afinidad se une puede ser agarosa, pero también están disponibles otras matrices. Las matrices mecánicamente estables, como vidrio poroso controlado o poli(estirenodivinil)benceno, permiten tasas de flujo más rápidas y tiempos de procesamiento más cortos que los que se pueden lograr con agarosa. Cuando el anticuerpo comprende un dominio CH3, la resina Bakerbond ABX™ (J. T. Baker, Phillipsburg, New Jersey) es útil para la purificación. También están disponibles, dependiendo del anticuerpo que quiera recuperarse, otras técnicas para la purificación de las proteínas, como fraccionamiento en una columna de intercambio iónico, precipitación en etanol, HPLC de fase inversa, cromatografía en anhídrido silícico, cromatografía en heparina SEPHAROSE™, cromatografía en una resina de intercambio iónico o catiónico (como una columna de ácido poliaspártico), cromatoisoenfoque, SDS-PAGE y precipitación en sulfato de amonio.

[0388] Siguiendo cualquiera de las fases preliminares de purificación, la mezcla que contiene el anticuerpo de interés y los contaminantes puede estar sujeta a nuevas etapas de purificación, por ejemplo, mediante cromatografía de interacción hidrófoba con pH bajo utilizando una solución de elución a un pH entre 2,5 y 4,5, generalmente a concentraciones bajas en sal (por ejemplo, entre 0-0,25M aproximadamente).

[0389] En general, están consolidados en la materia diversos métodos para la preparación de anticuerpos para uso en investigación, pruebas y uso clínico, son coherentes con todo lo anterior y/o se consideran apropiados por un experto en la materia para un anticuerpo concreto de interés.

Inmunoconjugados

[0390] La invención también proporciona inmunoconjugados (también denominados "conjugados de anticuerpos con fármacos" o "ADC"), que comprenden cualquiera de los anticuerpos anti-STEAP-1 de la invención conjugado a uno o más agentes citotóxicos como por ejemplo un agente quimioterapéutico, un fármaco, un agente inhibidor del crecimiento, una toxina (por ejemplo, una toxina enzimáticamente activa de origen bacteriano, fúngico, animal o vegetal, o fragmentos de la misma) o un isótopo radioactivo (es decir, un radioconjugado).

[0391] En ciertas formas de realización, un inmunoconjugado comprende un anticuerpo anti-STEAP-1 y un agente quimioterapéutico u otra toxina. Los agentes quimioterapéuticos útiles en la generación de inmunoconjugados se describen en la presente memoria descriptiva (por ejemplo, anteriormente). También se pueden utilizar toxinas enzimáticamente activas y fragmentos de las mismas y se describen en la presente memoria descriptiva.

[0392] En ciertas formas de realización, un inmunoconjugado comprende un anticuerpo anti-STEAP-1 y una o más toxinas de molécula pequeña, incluyendo, entre otros, fármacos de molécula pequeña como caliqueamicina, maitansinoide, dolastatina, auristatina, tricoteceno y CC1065, así como los derivados de estos fármacos que tengan actividad citotóxica. A continuación, se describen detalladamente ejemplos de estos inmunoconjugados.

1. Inmunoconjugados ejemplares

[0393]. Un inmunoconjugado (o "conjugado de anticuerpo con fármacos" ("ADC")) de la invención puede ser la Fórmula I, que figura a continuación, donde un anticuerpo anti-STEAP-1 se conjuga (es decir, se une covalentemente) con una o más fracciones de fármaco (D) a través de un enlazador opcional (L).

$$Ab–(L–D)_p \qquad \text{Fórmula I}$$

[0394]. Por consiguiente, el anticuerpo anti-STEAP-1 se puede conjugar con el fármaco ya sea directamente o mediante un enlazador. En la Fórmula I, p es el promedio de fracciones de fármaco por anticuerpo, que puede oscilar, por ejemplo, entre aproximadamente 1 y aproximadamente 20, fracciones de fármaco por anticuerpo y, en ciertas formas de realización, entre 1 y aproximadamente 8 fracciones de fármaco por anticuerpo.

Enlazadores ejemplares

[0395]. En la presente memoria descriptiva se detallan enlazadores y fracciones de fármacos ejemplares. Un enlazador puede comprender uno o más componentes enlazadores. Los componentes de enlace ejemplares incluyen: 6-maleimidocaproilo ("MC"), maleimidopropanoilo ("MP"), valina-citrulina ("val-cit" o "vc"), alanina-fenilalanina ("ala-phe"), p-aminobensiloxicarbonil ("PAB"), N-Succinimidil 4-(2-piridiltio) pentanoato ("SPP"), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato ("SMCC'), N-Succinimidil (4-yodo-acetil) aminobenzoato ("SIAB"), y etileneoxi-CH_2CH_2O- como una o más unidades repetidoras ("EO" o "PEO"). Diversos componentes enlazadores son conocidos en la materia y algunos se describen más adelante.

[0396]. Un enlazador puede ser un "enlazador escindible", que facilite la liberación de un fármaco en la célula. Por ejemplo, puede utilizarse un enlazador lábil al ácido (por ejemplo, hidrazona), un enlazador sensible a la proteasa (por ejemplo, sensible a la peptidasa), un enlazador fotolábil, un enlazador de dimetilo o un enlazador que contenga disulfuro (Chari y col., *Cancer Research* 52:127131 (1992); patente de EE.UU. N.º 5.208.020).

[0397]. En una forma de realización, el enlazador L de un ADC posee la fórmula:

$$—A_a—W_w—Y_y—$$

en la que:

-A- es una unidad de extensión unida covalentemente a un tiol de cisteína del anticuerpo (Ab),

a es 0 ó 1,

cada -W- es independientemente una unidad de aminoácidos,

w es independientemente un número entero que oscila entre 0 y 12,

-Y- es una unidad espaciadora unida covalentemente a una fracción de fármaco e

y es 0, 1 ó 2.

Unidad de extensión

[0398]. La unidad de extension (-A-), cuando está presente, es capaz de unir una unidad de anticuerpos a una unidad de aminoácidos (-W-). Al respecto, un anticuerpo (Ab) posee un grupo tiol de cisteína libre que puede formar un enlace con un grupo electrofílico funcional de una unidad de extensión. Las unidades de extensión ejemplares en los conjugados de Fórmula I se describen en las Fórmulas II y III, en las que Ab-, -W-, -Y-, -D, w e y son como se definió anteriormente y R^{17} es un radical divalente seleccionado de $(CH_2)_r$, C_3-C_8 carbociclilo, O–$(CH_2)_r$, arileno, $(CH_2)_r$–arileno, –arileno–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbociclilo), (C_3-C_8 carbociclilo)–$(CH_2)_r$, C_3-C_8 heterociclilo, $(CH_2)_r$–(C_3-C_8 heterociclilo), –(C_3-C_8 heterociclilo)–$(CH_2)_r$–, –$(CH_2)_rC(O)NR^b(CH_2)_r$–, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–,

$-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-$, $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$, $-(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r-$, $-(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$ y $-(CH_2CH_2O)_rC(O)NR^b(CH_2)_r-$; donde R^b es H, C_1-C_6 alquilo, fenilo o bencilo y r es independientemente un número entero que oscila entre 1 y 10.

[0399]. El arileno incluye radicales de hidrocarburo aromático divalente de entre 6 y 20 átomos de carbono derivados de la eliminación de dos átomos de hidrógeno del sistema del anillo aromático. Los grupos de arileno típicos incluyen, entre otros, radicales derivados del benceno, benceno sustituido, naftaleno, antraceno, bifenilo, y similares.

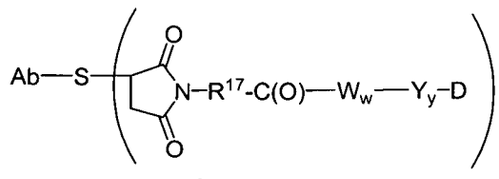
[0400]. Los grupos heterociclilos incluyen un sistema de anillos en el que uno o más átomos en anillo es un heteroátomo, por ejemplo, nitrógeno, oxígeno y sulfuro. El radical heterociclo comprende de 1 a 20 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S. Un heterociclo puede ser un monociclo con 3 a 7 anillos (de 2 a 6 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S) o un biciclo con 7 a 10 anillos (de 4 a 9 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S), por ejemplo: un sistema biciclo [4,5], [5,5], [5,6] o [6,6]. Los heterociclos se describen en Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* (W.A. Benjamin, New York, 1968), especialmente en los capítulos 1, 3, 4, 6, 7 y 9; *The Chemistry of Heterocyclic Compounds, A series of Monographs* (John Wiley y Sons, New York, 1950 a nuestros días), particularmente en los volúmenes 13, 14, 16, 19 y 28; y *J. Am. Chem. Soc.* (1960) 82:5566.

[0401]. Ejemplos de heterociclos son piridil, dihidroipiridil, tetrahidropiridil (piperidil), tiazolil, tetrahidrotiofeno, tetrahidrotiofeno de azufre oxidado, pirimidinil, furanil, tienil, pirrolil, pirazolil, imidazolil, tetrazolil, benzofuranil, tianaftalenil, indolil, indolenil, quinolinil, isoquinolinil, benzimidazolil, piperidinil, 4-piperidonil, pirrolidinil, 2-pirrolidonil, pirrolinil, tetrahidrofuranil, bis-tetrahidrofuranil, tetrahidropiranil, bis-tetrahidropiranil, tetrahidroquinolinil, tetrahidroisoquinolinil, decahidroquinolinil, octahidroisoquinolinil, azocinil, triazinil, 6H-1,2,5-tiadiazinil, 2H,6H-1,5,2-ditiazinil, tienil, tiantrenil, piranil, isobenzofuranil, chromenil, xantenil, fenoxatinil, 2H-pirrolil, isotiazolil, isoxazolil, pirazinil, piridazinil, indolizinil, isoindolil, 3H-indolil, 1H-indazolil, purinil, 4H-quinolizinil, ftalazinil, naftiridinil, quinoxalinil, quinazolinil, cinnolinil, pteridinil, 4Ah-carbazolil, carbazolil, β-carbolinil, fenantridinil, acridinil, pirimidinil, fenantrolinil, fenazinil, fenotiazinil, furazanil, fenoxazinil, isochromanil, chromanil, imidazolidinil, imidazolinil, pirazolidinil, pirazolinil, piperazinil, indolinil, isoindolinil, quinuclidinil, morfolinil, oxazolidinil, benzotriazolil, benzisoxazolil, oxindolil, benzoxazolinil e isatinoil, entre otros.

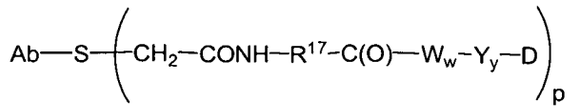
[0402]. Los grupos carbociclilos incluyen un anillo saturado o insaturado con entre 3 y 7 átomos de carbono como monociclo o entre 7 y 12 átomos de carbono como biciclo. Los carbociclos monociclicos tienen de 3 a 6 átomos en anillo y aún con más frecuencia 5 ó 6 átomos en anillo. Los carbociclos bicíclicos tienen de 7 a 12 átomos en anillo, por ejemplo, dispuestos como un sistema biciclo

[4,5], [5,5], [5,6] o [6,6] o de 9 a 10 átomos en anillo dispuestos como un sistema biciclo [5,6] o [6,6]. Ejemplos de carbociclos monocíclicos incluyen ciclopropil, ciclobutil, ciclopentil, 1-ciclopent-1-enil, 1-ciclopent-2-enil, 1-ciclopent-3-enil, ciclohexil, 1-ciclohex-1-enil, 1-ciclohex-2-enil, 1-ciclohex-3-enil, cicloheptil y ciclooctil.

[0403]. Se debe comprender a partir de todas las formas de realización ejemplares de ADC de Fórmula I , como II-VI, que aún cuando no se lo denote expresamente, entre 1 y 4 fracciones de fármacos se unen a un anticuerpo (p = 1-4), dependiendo de la cantidad de residuos diseñados con cisteína.

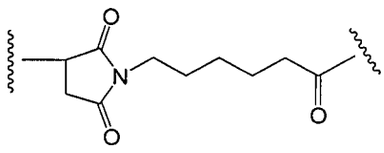


II



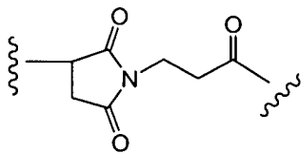
III

[0404]. Una unidad de extensión ilustrativa de Fórmula II deriva de maleimida-caproila (MC) donde R^{17} es $-(CH_2)_5-$:



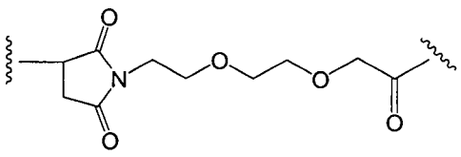
MC

[0405]. Una unidad de extensión ilustrativa de Fórmula II y deriva de maleimida-propanoilo (MP) donde R^{17} es $-(CH_2)_2-$:

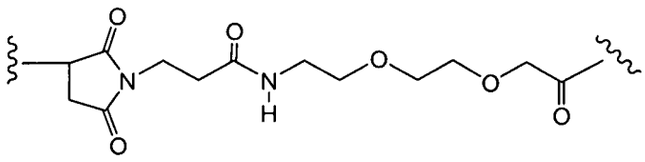


MP

[0406]. Otra unidad de extensión ilustrativa de Fórmula II en la que R^{17} es $-(CH_2CH_2O)_r-CH_2-$ y r es 2:

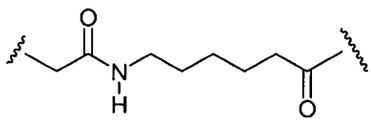


[0407]. Otra unidad de extensión ilustrativa de Fórmula II en la que R^{17} es $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$ donde R^b es H y cada r es 2:

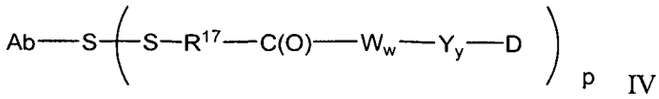


MPEG

[0408]. Una unidad de extensión ilustrativa de Fórmula III en la que R^{17} es $-(CH_2)_5-$:

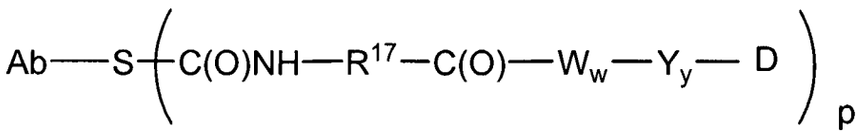


[0409]. En otra forma de realización, la unidad de extensión se une al anticuerpo anti-STEAP-1 diseñado con cisteína mediante un enlace disulfuro entre el átomo de sulfuro diseñado con cisteína del anticuerpo y un átomo de sulfuro de la unidad de extensión. Una unidad de extensión representativa de esta forma de realización se describe en la Fórmula IV, en la que R^{17}, Ab-, -W-, -Y-, -D, w e y son tal cual se definieron anteriormente.

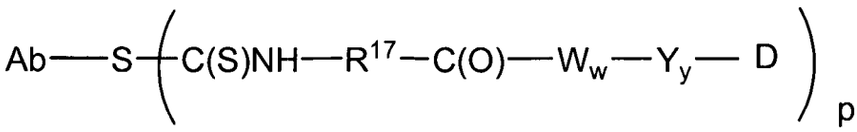


IV

[0410]. En otra forma de realización, el grugo reactivo de la unidad de extensión contiene un grupo funcional de reactivos de tiol que pueden formar un enlace con un tiol de cisteína libre de un anticuerpo. Entre los ejemplos de los grupos funcionales que reaccionan con el tiol se pueden mencionar, entre otros, la maleimida, α-haloacetil, éteres activados como los ésteres de succinimida, ésteres de 4-nitrofenil, ésteres de pentafluorofenil, ésteres de tetrafluorofenil, anhídridos, cloruros de ácidos, fluoruro sulfonil, isocianatos e isotiocianatos. Unidades de extensión representativas de esta forma de realización se describen en la Fórmula Va y Vb en la que R^{17}-, Ab-, -W-, -Y-, -D, w e y son tal cual se definieron anteriormente.



Va



Vb

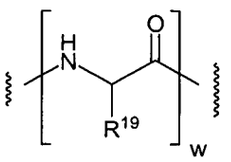
[0411]. En otra forma de realización, el enlazador puede ser de tipo dendrítico para facilitar el enlace covalente de más de una fracción de fármacos a un anticuerpo mediante una fracción de enlazador multifuncional ramificado (Sun y col., (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215;

Sun y col., (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768; King (2002) *Tetrahedron Letters* 43:1987-1990). Los enlazadores dendríticos pueden incrementar la proporción molar de un fármaco a un anticuerpo, es decir, la concentración, que se relaciona con la potencia del ADC. Por lo tanto, cuando un conjugado de anticuerpos con fármacos sólo contiene un grupo de tioles de cisteína reactiva, se pueden unir muchísimas fracciones de fármaco a través de un enlazador dendrítico.

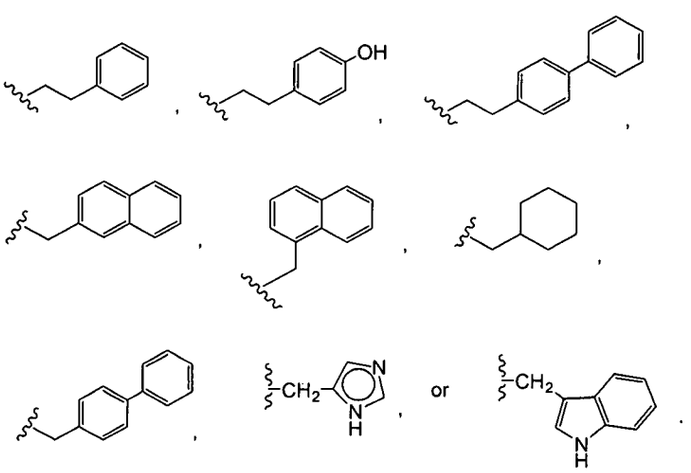
Unidad de aminoácidos

[0412]. El enlazador puede incluir residuos de aminoácidos. La unidad de aminoácidos (-W_w-), cuando está presente, une el anticuerpo (Ab) con la fracción de fármaco (D) del conjugado de anticuerpo diseñado con cisteína con fármacos (ADC) de la invención.

[0413]. -W_w- es una unidad dipéptida, tripéptida, tetrapéptida, pentapéptida, hexapéptida, heptapéptida, octapéptida, nonapéptida, decapéptida, undecapéptida o dodecapéptida. Los residuos de aminoácidos que comprenden la unidad de aminoácidos incluyen aquellos que se producen de forma natural, así como también los aminoácidos menores y los análogos de aminoácidos que no se producen de forma natural, como la citrulina. Cada unidad -W- tiene independientemente la fórmula que se muestra a continuación entre corchetes; w es un número entero que oscila entre 0 y 12.



donde R^{19} es hidrógeno, metilo, isopropilo, isobutilo, secbutil, bencilo, p-hidroxibencilo, $-CH_2OH$, $-CH(OH)CH_3$, $-CH_2CH_2SCH_3$, $-CH_2CONH_2$, $-CH_2COOH$, $-CH_2CH_2CONH_2$, $-CH_2CH_2COOH$, $-(CH_2)_3NHC(=NH)NH_2$, $-(CH_2)_3NH_2$, $-(CH_2)_3NHCOCH_3$, $-(CH_2)_3NHCHO$, $-(CH_2)_4NHC(=NH)NH_2$, $-(CH_2)_4NH_2$, $-(CH_2)_4NHCOCH_3$, $-(CH_2)_4NHCHO$, $-(CH_2)_3NHCONH_2$, $-(CH_2)_4NHCONH_2$, $-CH_2CH_2CH(OH)CH_2NH_2$, 2-piridilmetil-, 3-piridilmetil-, 4-piridilmetil-, fenilo, ciclohexil,



[0414]. Cuando R^{19} es otro elemento que no sea hidrógeno, el átomo de carbono al cual R^{19} se une es quiral. Cada átomo de carbono al cual R^{19} se une es independiente en la configuración (S) o (R) o una mezcla racémica. Por lo tanto, las unidades de aminoácidos pueden ser enantioméricamente puras, racémicas o diastereoméricas.

[0415]. Las unidades de aminoácidos $-W_w-$ ejemplares incluyen un dipéptido, un tripéptido, un tetrapéptido o un pentapéptido. Los dipéptidos ejemplares incluyen: valina-citrulina (vc o val-cit), alanina-fenilalanina (af o ala-phe). Los tripéptidos ejemplares incluyen: glicina-valina-citrulina (gli-val-cit) y glicina-glicina-glicina (gli-gli-gli). Los residuos de aminoácidos que comprenden un componente enlazador de aminoácidos incluyen aquellos que se producen de forma natural, tales como los aminoácidos menores y los análogos de aminoácidos que no se producen de forma natural, como la citrulina.

[0416]. La unidad de aminoácidos puede separarse enzimáticamente por una o más enzimas, incluyendo una proteasa asociada a un tumor, para liberar la fracción de fármaco (-D), que en una forma de realización se le incorpora un protón *in vivo* en la liberación para proporcionar un fármaco (D). Los componentes enlazadores de aminoácidos pueden designarse y optimizarse en su selectividad para la escisión enzimática mediante una enzima específica, por ejemplo, una proteasa asociada a un tumor, catepsina B, C y D o una proteasa plasmina.

Unidad espaciadora

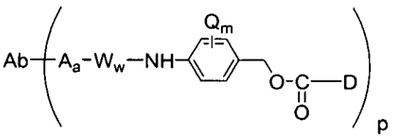
[0417]. La unidad espaciadora $(-Y_y-)$, cuando se encuentra presente (y = 1 ó 2), une una unidad de aminoácidos $(-W_w-)$ a una fracción de fármaco (D) cuando una unidad de aminoácidos se encuentra presente (w = 1-12). Alternativamente, la unidad espaciadora une la unidad de extensión a la fracción de fármaco cuando la unidad de aminoácidos no está presente. La unidad espaciadora también une la fracción de fármaco a la unidad de anticuerpos cuando la unidad de aminoácidos y la unidad de extensión no están presentes (w, y = 0). Las unidades espaciadoras son de dos tipos generales: autoinmoladoras y no autoinmoladoras. Una unidad espaciadora no autoinmoladora es aquella en la que la totalidad o parte de la unidad espaciadora se mantiene unida a la fracción de fármaco después de la escisión, particularmente enzimática, de una unidad de aminoácidos del conjugado de anticuerpos con fármacos o del enlazador de fracciones de fármaco. Cuando un ADC con una unidad espaciadora de glicina-glicina o una unidad espaciadora de glicina sufre una escisión enzimática a través de una proteasa asociada a una célula tumoral, una proteasa asociada a una célula cancerosa o una proteasa asociada a un linfocito, una fracción de fármaco de glicina-glicina o una fracción de fármaco de glicina se separa de Ab-A_a-Ww-. En una forma de realización, una reacción de hidrólisis independiente se produce dentro de la célula diana, lo cual separa el enlace de la fracción de fármaco de la glicina y libera el fármaco.

[0418]. En otra forma de realización, $-Y_y-$ es una unidad de p-aminobencilcarbamoil (PAB) cuya porción de fenileno se sustituye por Q_m, donde Q es $-C_1-C_8$ alquilo, $-O-(C_1-C_8$ alquilo), -halógeno,-nitro o -ciano y m es un número entero que oscila entre 0 y 4.

[0419]. Las formas de realización ejemplares de una unidad espaciadora no autoimmoladoras son: -Gly-Gly- ; -Gly- ; -Ala-Phe- ; -Val-Cit- .

[0420]. En una forma de realización, se proporciona un enlazador de fracciones de fármaco o un ADC en el que la unidad espaciadora está ausente (y=0) o está una de sus sales o disolventes farmacéuticamente aceptables.

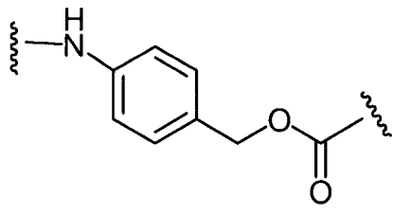
[0421]. Alternativamente, un ADC que contiene una unidad espaciadora autoinmoladora puede liberar –D. En una forma de realización, -Y- es un grupo PAB unido a $-W_w-$ a través del átomo de nitrógeno del amino del grupo PAB y está conectado directamente a –D a través de un carbonato, carbamato o grupo de éteres, donde el ADC posee la estructura ejemplar:



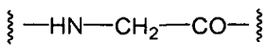
donde Q es $-C_1-C_8$ alquilo, $-O-(C_1-C_8$ alquilo), -halógeno, -nitro o -ciano; m es un número entero que oscila entre 0 y 4, y p oscila entre 1 y 4.

[0422]. Otros ejemplos de espaciadores autoinmoladores incluyen, entre otros, compuestos aromáticos que se asemejan electrónicamente al grupo PAB, como los derivados del 2-aminoimidazol-5-metanol (Hay y col., (1999) *Bioorg. Med. Chem. Lett.* 9:2237), análogos de PAB heterocíclicos (patente de EE.UU. N.º 2005/0256030), beta-glucorónido (documento WO 2007/011968) y orto- o para-aminobencilacetales. Se pueden utilizar espaciadores que se sometan a ciclización tras hidrólisis de la unión amida, como amidas de ácido 4-aminobutírico sustituido y no sustituido (Rodrigues y col., (1995) *Chemistry Biology* 2: 223); sistemas de anillo biciclo[2.2.1] y biciclo[2.2.2] sustituidos adecuadamente (Storm y col. (1972) *J. Amer. Chem. Soc.*, 94:5815) y amidas de ácido 2-aminofenilpropiónico (Amsberry y col., (1990) *J. Org. Chem.*, 55:5867). La eliminación de fármacos con aminas que se sustituyen en la glicina (Kingsbury y col. (1984) *J. Med. Chem.* 27:1447) también es un ejemplo de espaciadores autoinmoladores útiles en los ADC.

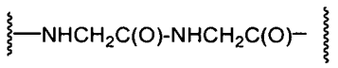
[0423]. Las Fórmulas X-XII representan las unidades espaciadoras ejemplares ($-Y_y-$).



X



XI

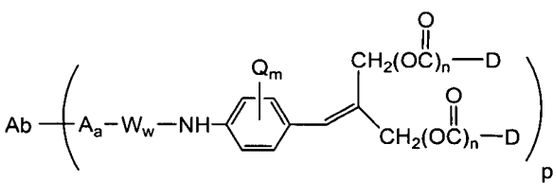


XII

Enlazadores dendríticos

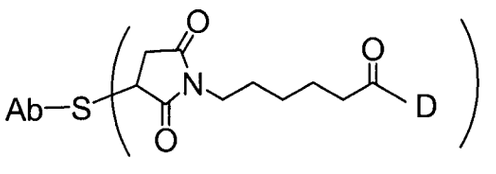
[0424]. En otra forma de realización, el enlazador L puede ser de tipo dendrítico para facilitar el enlace covalente de más de una fracción de fármacos a un anticuerpo a través de una fracción de enlazador multifuncional ramificado (Sun y col., (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun y col., (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Los enlazadores dendríticos pueden incrementar la proporción molar de un fármaco a un anticuerpo, es decir, la concentración, que se relaciona con la potencia del ADC. Por lo tanto, cuando un conjugado de anticuerpos con fármacos sólo contiene un grupo de tioles de cisteína reactiva, se pueden unir muchísimas fracciones de fármaco a través de un enlazador dendrítico. Entre las formas de realización ejemplares de enlazadores dendríticos ramificados se puede mencionar 2,6-bis(hidroximetil)-p-cresol y unidades de dendrímeros de 2,4,6-tris(hidroximetil)-fenol (documento WO 2004/01993; Szalai y col., (2003) *J. Amer. Chem. Soc.* 125:15688-15689; Shamis y col., (2004) *J. Amer. Chem. Soc.* 126:1726-1731; Amir y col., (2003) *Agnew. Chem. Int. Ed.* 42:4494-4499).

[0425]. En una forma de realización, una unidad espaciadora es una unidad ramificada de bis(hidroximetil)estireno (BHMS), que se puede utilizar para incorporar y liberar múltiples fármacos, que posean la siguiente estructura:

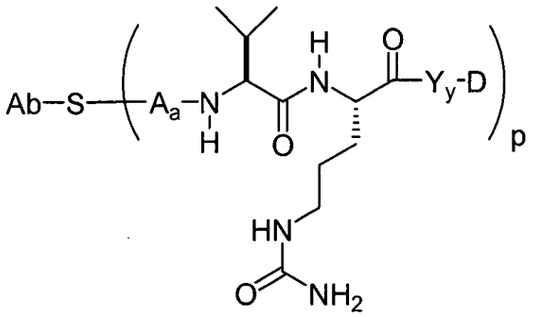


que compenda una unidad de dendrímeros de 2-(4-aminobencilideno)propano-1,3-diol (documento WO 2004/043493; de Groot y col., (2003) *Agnew. Chem. Int. Ed.* 42:4490-4494), donde Q es -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -halógeno, -nitro o -ciano; m es un número entero que oscila entre 0 y 4; n es 0 ó 1 y p oscila entre 1 y 4.

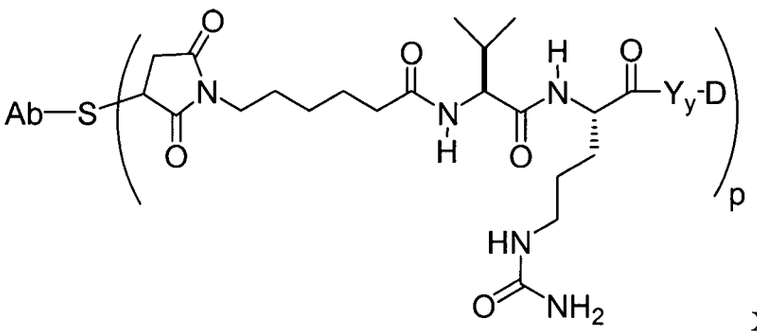
[0426]. Formas de realización ejemplares de los compuestos de conjugados de anticuerpos con fármacos de Fórmula I incluyen XIIIa (MC), XIIIb (val-cit), XIIIc (MC-val-cit) y XIIId (MC-val-cit-PAB):



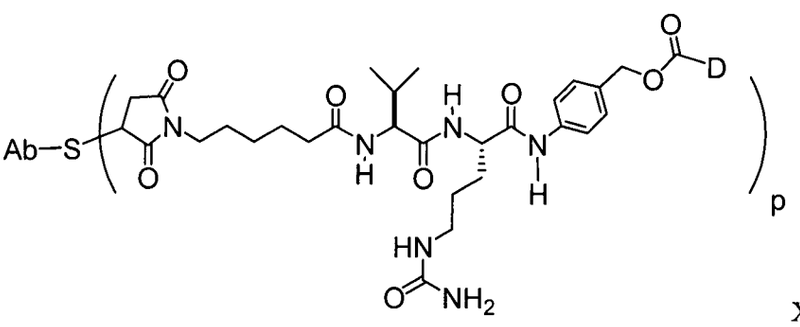
XIIIa



XIIIb

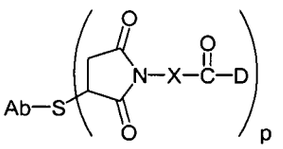


XIIIc

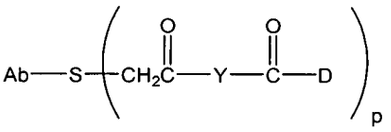


XIIId

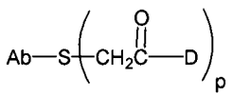
[0427]. Otras formas de realización ejemplares de los compuestos de conjugados de anticuerpos con fármacos de Fórmula Ia incluyen XIV a-e:



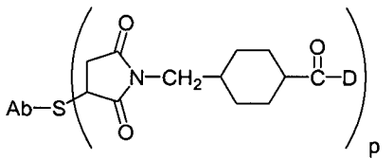
XIVa



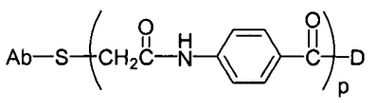
XIVb



XIVc

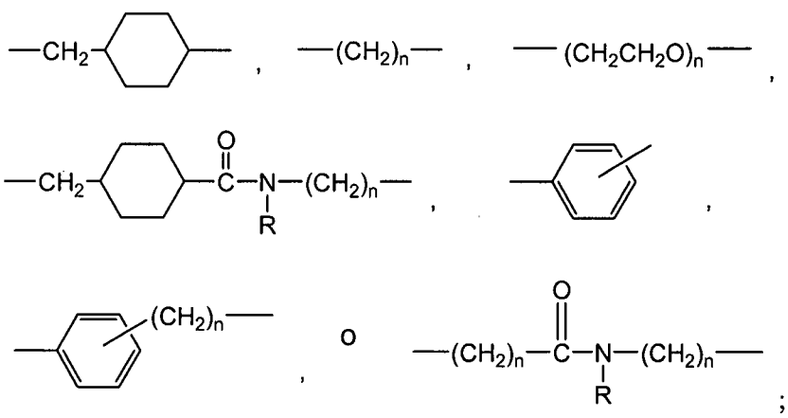


XIVd

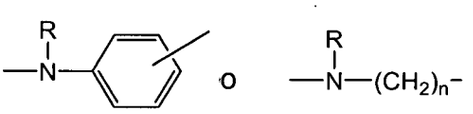


XIVe

donde X es:



Y es:



y R es independientemente H o C_1–C_6 alquilo y n oscila entre 1 y 12.

[0428]. En otra forma de realización, un enlazador posee un grupo funcional reactivo que posee un grupo nucleofílico reactivo a un grupo electrofílico presente en un anticuerpo. Algunos de los grupos electrofílicos útiles en un anticuerpo incluyen, entre otros, los grupos carbonilos de aldehídos y acetonas. El heteroátomo de un grupo nucleofílico de un enlazador puede reaccionar con un grupo electrofílico en un anticuerpo y formar un enlace covalente a una unidad de anticuerpos. Entre los grupos nucleofílicos útiles en un enlazador se puede mencionar la hidracida, oxima, amina, hidracina, tiosemicarbazona, carboxilato de hidracina y aril-hidracida. El grupo electrofílico en un anticuerpo proporciona un sitio conveniente para la unión con un enlazador.

[0429]. Por lo general, los enlazadores tipo péptidos pueden prepararse formando un enlace peptídico entre uno o más aminoácidos o fragmentos de péptidos. Estos enlaces peptídicos pueden prepararse, por ejemplo, de acuerdo con el método de síntesis de fase líquida (véase E. Schröder y K. Lübke (1965) *The Peptides*, volumen 1, págs. 76-136, Academic Press) que es bien conocido en el campo de la química de péptidos. Los enlazadores intermedios pueden ensamblarse con cualquier combinación o secuencia de reacciones incluyendo unidades espaciadoras, de extensión y de aminoácidos. Las unidades espaciadoras, de extensión y de aminoácidos pueden utilizar grupos funcionales reactivos que sean de naturaleza electrofílicos, nuclefílicos o sin radicales. Los grupos funcionales reactivos incluyen, entre otros, los carboxilos, hidroxilos, para-nitrofenilcarbonato, isotiocianato y grupos salientes como O-mesil, O-tosil, -Cl, -Br, -I o maleimida.

[0430]. En otra forma de realización, el enlazador puede sustituirse con grupos que modularon la solubilidad o reactividad. Por ejemplo, un sustituto cargado, como el sulfonato ($-SO_3^-$) o amonio, puede incrementar la solubilidad en agua del reactivo y facilitar la reacción de unión del reactivo enlazador con el anticuerpo o la fracción de fármacos, o facilitar la reacción de unión de Ab-L (enlazador a anticuerpo intermedio) con D o D-L (enlazador a fármaco intermedio) con Ab, dependiendo de la ruta sintética empleada para preparar el ADC.

Fracciones de fármacos ejemplares

Maitansina y maitansinoides

[0431]. En algunas realizaciones, un inmunoconjugado comprende un anticuerpo de la invención conjugado con una o más moléculas de maitansinoides. Los maitansinoides son inhibidores mitóticos que actúan inhibiendo la polimerización de la tubulina. La maitansina se aisló por primera vez a partir del arbusto de África oriental *Maytenus serrata* (patente de EE.UU. nº 3896111). Posteriormente, se descubrió que determinados microbios también producían maitansinoides, como el maitansinol y los ésteres de C-3 maitansinol (patente de EE.UU. nº 4.151.042). El maitansinol sintético y sus derivados y análogos se detallan, por ejemplo, en las patentes de EE.UU. Nºs. 4.137.230; 4.248.870; 4.256.746; 4.260.608; 4.265.814; 4.294.757; 4.307.016; 4.308.268; 4.308.269; 4.309.428; 4.313.946; 4.315.929; 4.317.821; 4.322.348; 4.331.598; 4.361.650; 4.364.866; 4.424.219; 4.450.254; 4.362.663 y 4.371.533.

[0432]. Las fracciones de fármacos maitansinoides son fracciones de fármacos que resultan atractivas para la elaboración de conjugados de anticuerpos con fármacos por sus siguientes características: (i) son relativamente accesibles en lo que se refiere a su preparación mediante fermentación, modificación química o derivación de productos de fermentación, (ii) son fáciles de someter a derivación, con grupos funcionales adecuados para conjugación a través de los ligandos no disulfuro con anticuerpos, (iii) estabilidad en plasma, y (iv) resultan efectivos frente a distintas líneas celulares tumorales.

[0433]. Los compuestos de maitansina aptos para uso como fracciones de fármacos maitansinoides son bien conocidos en el sector y se pueden aislar de fuentes naturales según métodos conocidos o se pueden producir mediante técnicas de ingeniería genética (véase Yu y col. (2002) *PNAS* 99:7968-7973). El maitansinol y los análogos del maitansinol también se pueden preparar de forma sintética mediante métodos conocidos.

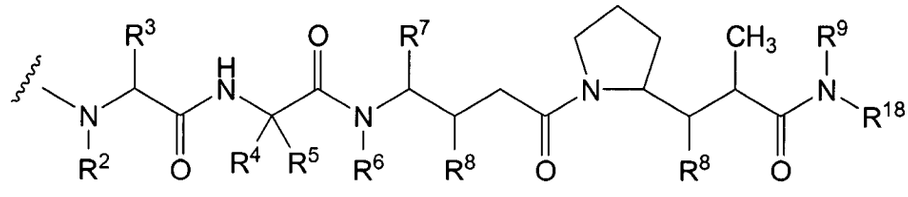
[0434]. A continuación se citan algunos ejemplos de formas de realización ejemplares de fracciones de fármacos maitansinoides: DM1; DM3; y DM4, tal como se describe en la presente memoria.

Auristatinas y dolastatinas

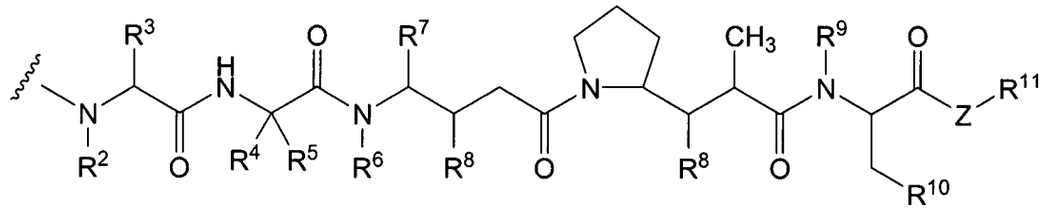
[0435]. En algunas formas de realización, un inmunoconjugado comprende un anticuerpo de la invención conjugado con dolastatina o un análogo o derivado peptídico de la dolastatina, por ejemplo, una auristatina (Patentes de EE.UU. núms. 5635483; 5780588). Se ha demostrado que las dolastatinas y las auristatinas interfieren con la dinámica de microtúbulos, la hidrólisis GTP, y la división nuclear y celular (Woyke y col. (2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) y tiene actividad antineoplásica (Patente de EE.UU. n.º5663149) y antifúngica (Pettit y col. (1998) *Antimicrob. Agents Chemother.* 42:2961-2965). La porción del fármaco auristatina o dolastatina puede estar unida al anticuerpo a través de los extremos terminales N (amino) o C (carboxil) de la porción peptídica del fármaco (WO 02/088172).

[0436]. Entre las formas de realización ejemplares de auristatina están las fracciones DE y DF de fármacos de monometilauristatina enlazados al extremo N-terminal, descritas en Senter y col., *Proceedings of the American Association for Cancer Research*, Volume 45, Abstract Number 623, presentada el 28 de marzo de 2004, cuyo contenido se incluye expresamente en su totalidad a modo de referencia.

[0437]. Una fracción peptídica de un fármaco puede seleccionarse entre las Fórmulas D_E and D_F siguientes:



D_E



D_F

donde la línea ondulada de D_E y D_F indica el punto de unión covalente a un anticuerpo o a un componente ligando-anticuerpo, e independientemente en cada ubicación:

R^2 se selecciona entre H y alquilo C_1-C_8;

R^3 se selecciona entre H, C_1-C_8 alquilo, C_3-C_8 carbociclo, arilo, C_1-C_8 alquilo-arilo, C_1-C_8 alquilo-(C_3-C_8 carbociclo), C_3-C_8 heterociclo y C_1-C_8 alquilo-(C_3-C_8 heterociclo);

R^4 se selecciona entre H, C_1-C_8 alquilo, C_3-C_8 carbociclo, arilo, C_1-C_8 alquilo-arilo, C_1-C_8 alquilo-(C_3-C_8 carbociclo), C_3-C_8 heterociclo y C_1-C_8 alquilo-(C_3-C_8 heterociclo);

R^5 se selecciona entre H y metilo;

o R^4 y R^5 forman conjuntamente un anillo carbocíclico y tienen la fórmula $-(CR^aR^b)_n-$ donde R^a y R^b se seleccionan independientemente entre H, alquilo C_1-C_8 y carbociclo C_3-C_8, y n se selecciona entre 2, 3, 4, 5 y 6;

R^6 se selecciona entre H y C_1-C_8 alquilo;

R^7 se selecciona entre H, C_1-C_8 alquilo, C_3-C_8 carbociclo, arilo, C_1-C_8 alquilo-arilo, C_1-C_8 alquilo-(C_3-C_8 carbociclo), C_3-C_8 heterociclo y C_1-C_8 alquilo-(C_3-C_8 heterociclo);

cada R^8 se selecciona independientemente entre H, OH, C_1-C_8 alquilo, C_3-C_8 carbociclo y O-(C_1-C_8 alquilo);

R^9 se selecciona entre H y C_1-C_8 alquilo;

R^{10} se selecciona entre arilo o C_3-C_8 heterociclo;

Z es O, S, NH o NR^{12}, donde R^{12} es C_1-C_8 alquilo;

R^{11} se selecciona entre H, alquilo C_1-C_{20}, arilo, heterociclo C_3-C_8, $-(R^{13}O)_m-R^{14}$, o $-(R^{13}O)_m-CH(R^{15})_2$;

m es un entero comprendido entre 1 y 1.000;

R^{13} es C_2-C_8 alquilo;

R^{14} es H o C1-C_8 alquilo;

cada R^{15} es independientemente H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, o $-(CH_2)_n-SO_3-C_1-C_8$ alquilo;

cada R^{16} es independientemente H, C_1-C_8 alquilo, o $-(CH_2)_n-COOH$;

R^{18} se selecciona entre $-C(R^8)_2-C(R^8)_2-$arilo, $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 heterociclo), y $-C(R^8)_2-C(R^8)_2-$(C_3-C_8 carbociclo); y

n es un número entero comprendido entre 0 y 6.

[0438]. En una forma de realización R^3, R^4 y R^7 son independientemente isopropilo o sec-butilo y R^5 es –H o metilo. En una forma ejemplar de realización, R^3 y R^4 son isopropilo, R^5 es -H, y R^7 es sec-butilo.

[0439]. En otra forma de realización, R^2 y R^6 son metilo y R^9 es -H.

[0440]. En otra forma de realización, cada R^8 es $-OCH_3$.

[0441]. En una forma ejemplar de realización, R^3 y R^4 son isopropilo, R^2 y R^6 son metilo, R^5 es -H, R^7 es sec-butilo, cada R^8 es $-OCH_3$ y R^9 es -H.

[0442]. En una forma de realización, Z es -O- o -NH-.

[0443]. En una forma de realización, R^{10} es arilo.

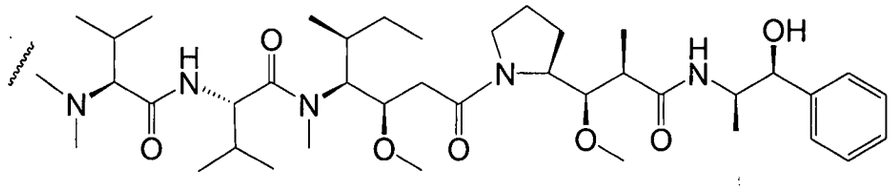
[0444]. En una forma ejemplar de realización, R^{10} es -fenilo.

[0445]. En una forma ejemplar de realización, cuando Z es -O-, R^{11} es –H, metilo o t-butilo.

[0446]. En una forma de realización, cuando Z es -NH, R^{11} es $-CH(R^{15})_2$, donde R^{15} es $-(CH_2)_n-N(R^{16})_2$, y R^{16} es $-C_1-C_8$ alquilo o $-(CH_2)_n-COOH$.

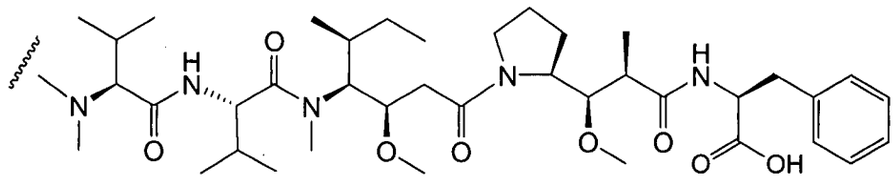
[0447]. En otra forma de realización, cuando Z es -NH, R^{11} es $-CH(R^{15})_2$, donde R^{15} es $-(CH_2)_n-SO_3H$.

[0448]. Una forma ejemplar de realización con auristatina de la fórmula D_E es MMAE, donde la línea ondulada indica el enlace covalente a un ligando (L) de un conjugado de anticuerpo con fármaco:



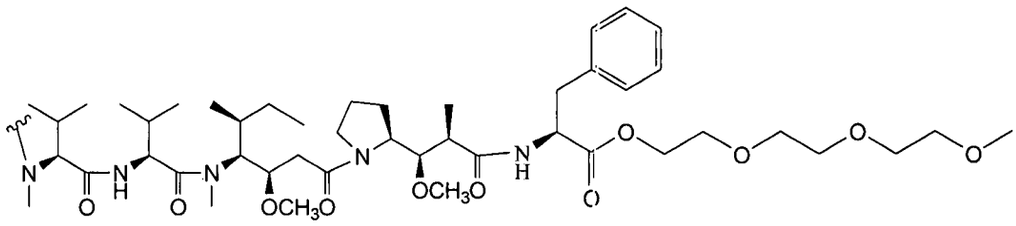
MMAE

[0449]. Una forma ejemplar de realización con auristatina de la fórmula D_F es MMAF, donde la línea ondulada indica el enlace covalente a un ligando (L) de un conjugado de anticuerpo con fármaco (véase US 2005/0238649 y Doronina y col. (2006) *Bioconjugate Chem.* 17:114-124):

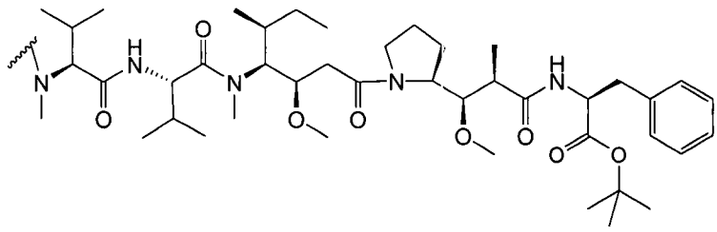


MMAF

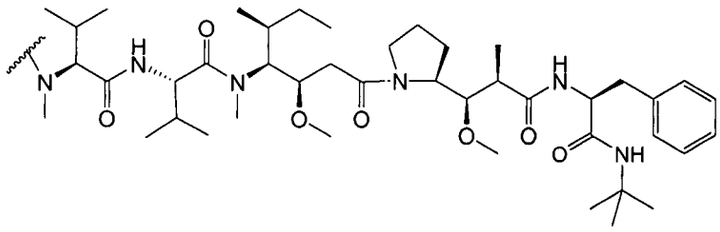
[0450]. Otras fracciones de fármaco incluyen los siguientes derivados de MMAF, donde la línea ondulada indica el enlace covalente a un ligando (L) de un conjugado de anticuerpo con fármaco:



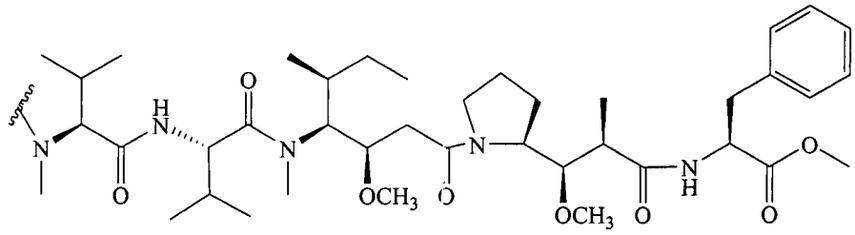
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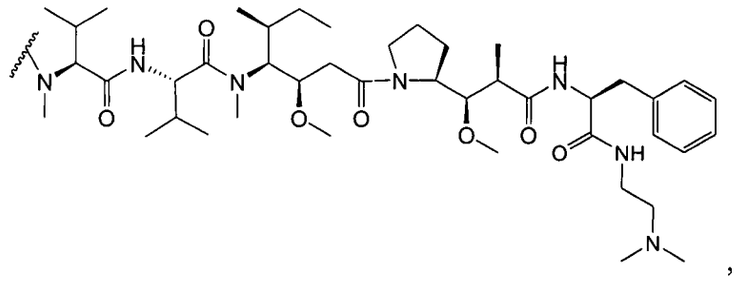
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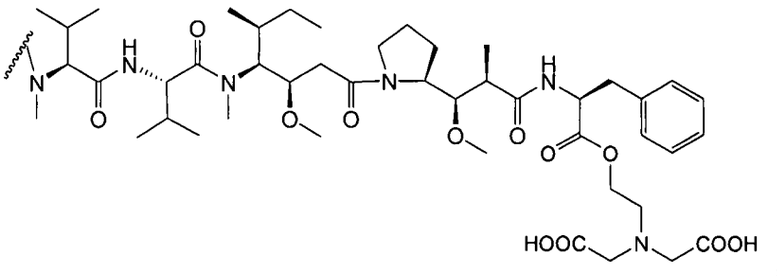
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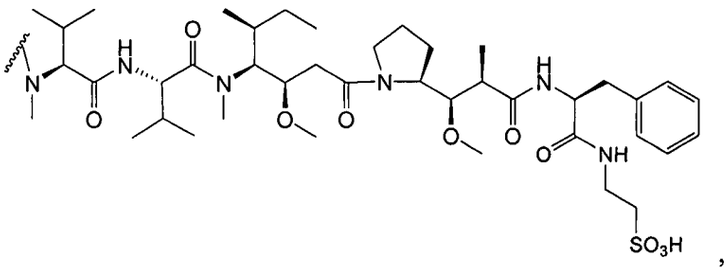
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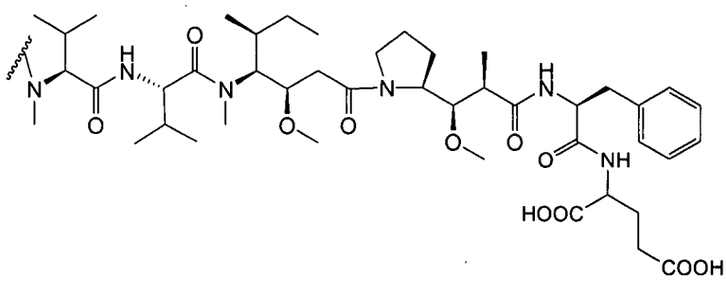
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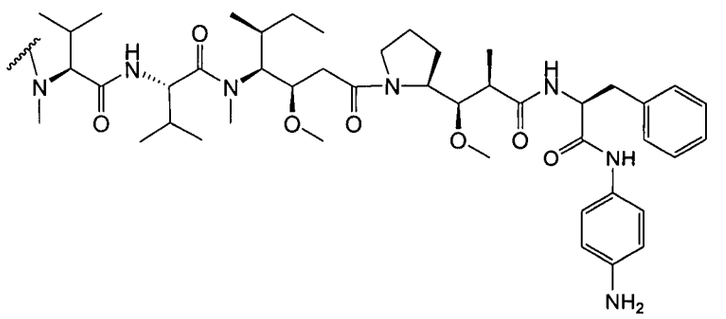
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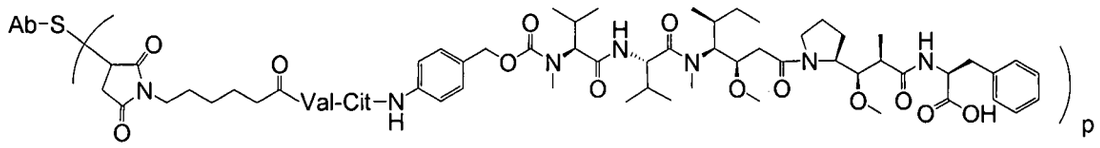
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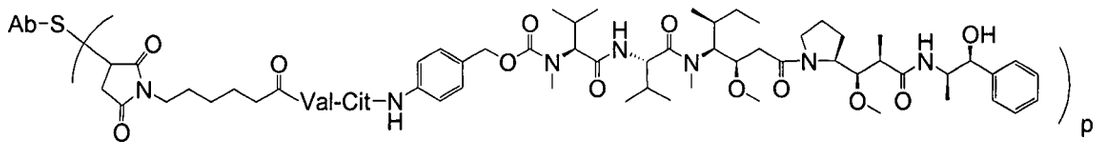
[0451]. En un aspecto, los grupos hidrofílicos que incluyen ésteres de glicol trietileno (TEG), como se muestra anteriormente, se pueden unir a la fracción del fármaco en R^{11}. Sin estar obligados por una teoría concreta, los grupos hidrofílicos ayudan a la internalización y no aglomeración de la fracción del fármaco.

[0452]. Las formas ejemplares de realización de los ADC de la Fórmula I que comprenden una auristatina/dolastatina o un derivado de la misma se describen en US 2005-0238649 A1 y Doronina y col.

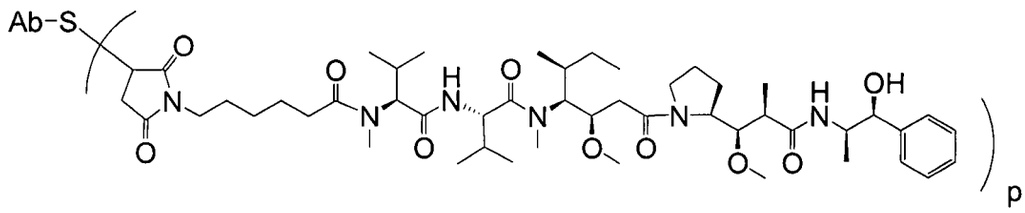
(2006) *Bioconjugate Chem.* 17:114-124, que se incluye expresamente en la presente memoria descriptiva a modo de referencia. Formas ejemplares de realización de los ADC de Fórmula I que comprenden MMAE o MMAF y diversos componentes ligandos tienen las siguientes estructuras y abreviaciones (donde "Ab" es un anticuerpo; p está comprendido entre 1 y 8 aproximadamente, "Val-Cit" es un dipéptido valina-citrulina; y "S" es un átomo de azufre:



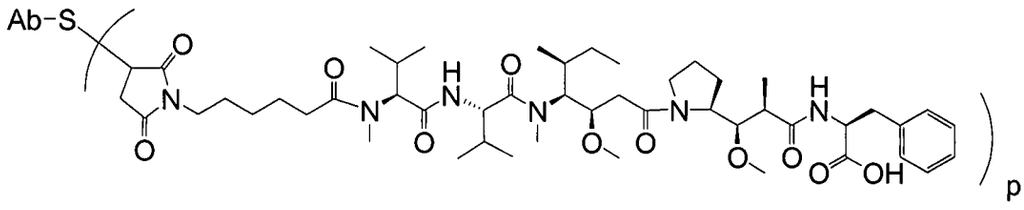
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

[0453]. Las formas de realización ejemplares de los ADC de la Fórmula I que comprenden MMAF y varios componentes ligandos incluyen, además, Ab-MC-PAB-MMAF y Ab-PAB-MMAF. Es interesante señalar que se ha demostrado que los inmunoconjugados que comprenden MMAF unido a un anticuerpo mediante un ligando que no sea proteolíticamente escindible poseen una actividad similar a la de los inmunoconjugados que comprenden MMAF unido a un anticuerpo mediante un ligando proteolíticamente escindible. Véase Doronina y col. (2006) *Bioconjugate Chem.* 17:114-124. En estas

sustancias, se cree que la liberación del fármaco se efectúa mediante la degradación del anticuerpo en la célula.

[0454]. Por lo general, las porciones de fármacos basadas en péptidos pueden prepararse formando un enlace peptídico entre uno o más aminoácidos y/o fragmentos de péptidos. Estos enlaces peptídicos pueden prepararse, por ejemplo, de acuerdo con el método de síntesis de fase líquida (véase E. Schröder y K. Lübke, "The Peptides", volume 1, págs. 76-136, 1965, Academic Press) que es bien conocido en el campo de la química de péptidos. Las porciones de fármacos auristatina/dolastatina pueden prepararse de acuerdo con los métodos siguientes: US 2005-0238649 A1; Patente de EE.UU. n.º 5635483; Patente de EE.UU. n.º 5780588; Pettit y col. (1989) *J. Am. Chem. Soc.* 111:5463-5465; Pettit y col. (1998) *Anti-cáncer Drug Design* 13:243-277; Pettit, G.R., y col. *Synthesis*, 1996, 719-725; Pettit y col (1996) *J. Chem. Soc. Perkin Trans.* 1 5:859-863; y Doronina (2003) *Nat. Biotechnol.* 21(7):778-784.

[0455]. En concreto, las fracciones de fármacos auristatina/dolastatina de la fórmula D_F, como MMAF y derivados de la misma, se pueden preparar usando métodos descritos en US 2005-0238649 A1 y Doronina y col. (2006) *Bioconjugate Chem.* 17:114-124. Las fracciones de fármacos auristatina/dolastatina de la fórmula D_E, como MMAE y derivados de las mismas, se pueden preparar usando métodos descritos en Doronina y col. (2003) *Nat. Biotech.* 21:778-784. Las fracciones de ligando-fármaco MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF y MC-vc-PAB-MMAE se pueden sintetizar fácilmente mediante métodos habituales, por ejemplo, como se describe en Doronina y col. (2003) *Nat. Biotech.* 21:778-784, y la publicación de la Solicitud de Patente n.º US 2005/0238649 A1, y luego conjugar con un anticuerpo de interés.

Concentración del fármaco

[0456]. La concentración del fármaco se representa mediante p y es el promedio de fracciones de fármaco por anticuerpo en una molécula de la Fórmula I. La concentración del fármaco puede oscilar entre 1 y 20 fracciones de fármaco (D) por anticuerpo. Los ADC de la Fórmula I incluyen conjuntos de anticuerpos conjugados con una variedad de fracciones de fármaco, de 1 a 20. El promedio de fracciones de fármaco por anticuerpo en preparaciones de ADC procedentes de reacciones de la conjugación se puede determinar mediante medios convencionales, como espectroscopía de masas, ensayo ELISA y HPLC. También se puede determinar la distribución cuantitativa de ADC en función de p. En algunos ejemplos, la separación, purificación y determinación del ADC homogéneo, donde p es un valor determinado de ADC con otras concentraciones de fármaco, se puede lograr por métodos como HPLC de fase reversa o electroforesis.

[0457]. En el caso de algunos conjugados de anticuerpos con fármacos, p puede verse limitado por el número de sitios de unión en el anticuerpo. Por ejemplo, cuando la unión es un tiol de la cisteína, como en las formas de realización ejemplares anteriores, puede que un anticuerpo sólo tenga uno o varios

grupos tiol de la cisteína, o puede tener sólo uno o varios grupos tiol suficientemente reactivos a través de los cuales se puede unir un ligando. En algunas formas de realización, una concentración más alta de fármaco, por ejemplo, p > 5, puede causar agregación, insolubilidad, toxicidad o pérdida de permeabilidad celular de determinados conjugados de anticuerpos con fármacos. En determinadas formas de realización, la concentración de fármaco para un ADC de la invención varía de 1 a aproximadamente 8; de aproximadamente 2 a aproximadamente 6; de aproximadamente 3 a aproximadamente 5; de aproximadamente 3 a aproximadamente 4; de aproximadamente 3,1 a aproximadamente 3,9; de aproximadamente 3,2 a aproximadamente 3,8; de aproximadamente 3,2 a aproximadamente 3,7; de aproximadamente 3,2 a aproximadamente 3,6; de aproximadamente 3,3 a aproximadamente 3,8; de aproximadamente 3,3 a aproximadamente 3,7. En realidad, se ha demostrado que para determinados ADC, la proporción óptima de fracciones de fármaco por anticuerpo puede ser inferior a 8, y puede ser de aproximadamente 2 a aproximadamente 5. Véase 2005-0238649 A1 de EE.UU. (incluida aquí en su totalidad como referencia).

[0458]. En determinadas formas de realización, un número menor al máximo teórico de fracciones de fármacos se conjuga con un anticuerpo durante una reacción de conjugación. Un anticuerpo puede contener, por ejemplo, residuos de lisina que no reaccionan con el ligando-fármaco intermedio o el reactivo del ligando, como se señala más adelante. Por lo general, los anticuerpos no contienen muchos grupos tioles de la cisteína libres y reactivos que se puedan unir a una fracción de fármaco; de hecho, la mayoría de los residuos tiol de la cisteína en anticuerpos están en forma de puentes disulfuro. En determinadas formas de realización, un anticuerpo se puede reducir con un agente reductor como ditiotreitol (DTT) o tricarboniletilfosfina (TCEP), en condiciones totales o parciales de reducción, para generar grupos tiol de cisteína reactivos. En determinadas formas de realización, un anticuerpo está sujeto a condiciones desnaturalizantes para revelar grupos nucleofílicos reactivos como lisina o cisteína.

[0459]. La concentración (proporción fármaco/anticuerpo) de un ADC se puede controlar de distintas formas, por ejemplo: (i) limitando el exceso molar del ligando-fármaco intermedio o reactivo ligando con respecto al anticuerpo, (ii) limitando el tiempo o la temperatura de la reacción de la conjugación, (iii) mediante condiciones reductivas parciales o limitadas para la modificación del tiol de la cisteína, (iv) diseñando mediante técnicas recombinantes la secuencia de aminoácido del anticuerpo de manera que se modifique la cantidad y la posición de residuos de cisteína para controlar el número y/o la posición de los enlaces ligando-fármaco (como thioMab o thioFab preparado según se describe en la presente memoria y en WO2006/034488 (incluido aquí en su totalidad como referencia)).

[0460]. Debe entenderse que, cuando más de un grupo nucleofílico reacciona con un ligando-fármaco intermedio o a un reactivo ligando seguido por el reactivo de la fracción del fármaco, el producto resultante es una mezcla de compuestos de ADC con una distribución de una o más fracciones de fármaco enlazadas a un anticuerpo. El promedio de fármacos por anticuerpo se puede calcular a partir de la mezcla

mediante un ensayo ELISA dual con anticuerpos, que sea específico para el anticuerpo y específico para el fármaco. Las moléculas de ADC individuales pueden identificarse en la mezcla mediante espectroscopía de masas y separarse mediante HPLC, por ejemplo, cromatografía de interacción hidrófoba (véase, por ejemplo, Hamblett, K.J., y col., "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, Marzo 27-31, 2004, Proceedings of the AACR, Volume 45, Marzo 2004; Alley, S.C., et al., "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, Marzo 27-31, 2004, Proceedings of the AACR, Volume 45, Marzo 2004). En determinadas formas de realización, un ADC homogéneo con un único valor de concentración puede aislarse de la mezcla de la conjugación mediante electroforesis o cromatografía.

Algunos métodos de preparación de inmunoconjugados

[0461]. Un ADC de la formula I puede prepararse por varios medios empleando reacciones químicas orgánicas, condiciones y reactivos conocidos por los expertos, como: (1) una reacción de un grupo nucleofílico de un anticuerpo con un agente ligando bivalente, para formar Ab-L, mediante una unión bivalente, seguida de la reacción con una fracción del fármaco D; y (2) una reacción de un grupo nucleofílico de una fracción del fármaco con un reactivo ligando bivalente, para formar D-L, mediante un enlace covalente, seguido de la reacción con un grupo nucleofílico de un anticuerpo. Los métodos ejemplares de preparación de un ADC de la Fórmula I por medio de la última vía se describen en 20050238649-0238649 A1 de EE.UU., que se incluye expresamente en la presente memoria descriptiva a modo de referencia.

[0462]. Los grupos nucleofílicos en los anticuerpos incluyen, aunque sin limitarse a ellos: (i) grupos amino N-terminal, (ii) grupos amino de la cadena lateral, por ej. lisina, (iii) grupos tiol de la cadena lateral, por ej. cisteína, y (iv) grupos amino o hidroxilo de azúcares donde el anticuerpo es glicosilado. Los grupos amino, tiol e hidroxilo son nucleofílicos y son capaces de reaccionar para formar enlaces covalentes con grupos electrofílicos en las fracciones del ligando y los reactivos ligandos, que incluyen: (i) ésteres activos como los ésteres NHS, los ésteres HOBt, haloformos, y haluros de acilo; (ii) haluros de alquilo y bencilo como las haloacetamidas; (iii) aldehídos, cetonas, carboxilo y grupos maleimidos. Ciertos anticuerpos contienen bisulfuros de intercadena reducible, es decir, puentes de cisteína. Se puede hacer que los anticuerpos sean reactivos para la conjugación con reactivos del ligando mediante tratamiento con un agente reductor como DTT (ditiotreitol) o tricarboniletilfosfina (TCEP), de forma que el anticuerpo quede total o parcialmente reducido. Por lo tanto, cada puente de cisteína formará, en teoría, dos nucleófilos tiol reactivos. Los grupos nucleofílicos adicionales pueden introducirse en los anticuerpos a través de la modificación de las lisinas residuales, por ejemplo, mediante reacción de residuos de lisina con 2-iminotiolano (reactivo de Traut), lo que da lugar a la conversión de una amina en un tiol. Los grupos tiol

reactivos pueden ser introducidos en un anticuerpo (o un fragmento del mismo) mediante la introducción de uno, dos, tres, cuatro o más residuos de cisteína (por ej. preparando variantes de anticuerpos que comprendan uno o más residuos de aminoácidos de cisteínas no nativas).

[0463]. Los conjugados de anticuerpos con fármacos de la invención también se pueden producir mediante reacción entre un grupo electrofílico en un anticuerpo, como un grupo carbonilo cetona o aldehído, con un grupo nucleofílico en un fármaco o reactivo ligando. Entre los grupos nucleofílicos útiles en un reactivo ligando se incluyen, aunque sin limitarse a ellos, hidracida, oxima, amina, hidrazina, tiosemicarbazona, carboxilato de hidrazina y arilhidrazida. En una forma de realización, un anticuerpo se modifica para introducir fracciones electrofílicas que puedan reaccionar con sustituyentes nucleofílicos en el fármaco o reactivo ligando. En otra forma de realización, los azúcares de los anticuerpos glicosilados pueden oxidarse, por ej. con reactivos oxidantes de periodato, a fin de formar grupos cetonas o aldehídos, los cuales pueden reaccionar con el grupo amina de los reactivos ligandos o las fracciones del fármaco. Los grupos resultantes de imina base de Schiff pueden formar un enlace estable, o pueden reducirse, por ej. mediante los reactivos de hidruro de boro para formar enlaces amina estables. En una forma de realización, la reacción de la porción carbohidratada de un anticuerpo glicosilado, ya sea con galactosa oxidasa o con metaperiodato de sodio, puede producir grupos carbonilo (aldehídos y cetonas) en el anticuerpo que pueden reaccionar con los grupos apropiados en el fármaco (técnicas de bioconjugado de Hermanson). En otra forma de realización, los anticuerpos que contienen residuos de serina o treonina N-terminal pueden reaccionar con metaperiodato de sodio, lo que da lugar a la producción de un aldehído en lugar del primer aminoácido (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; 5362852 de EE.UU.). Se puede hacer que este aldehído reaccione con una fracción de fármaco o un nucleófilo ligando.

[0464]. Los grupos nucleofílicos en una fracción del fármaco incluyen los siguientes compuestos, aunque sin limitarse a ellos: los grupos amino, tiol, hidroxilo, hidracida, oxima, hidracina, tiosemicarbazona, carboxilato de hidracina y aril-hidracida, capaces de reaccionar para formar enlaces covalentes con grupos electrofílicos en las fracciones ligando y reactivos ligando, que incluyen: (i) ésteres activos como los ésteres NHS, los ésteres HOBt, haloformos, y haluro de acilo; (ii) haluros de alquilo y bencilo como las haloacetamidas; (iii) aldehídos, cetonas, carboxilo y grupos maleimidos.

[0465]. Los compuestos de la invención contemplan expresamente el ADC preparado con los reactivos reticulares siguientes: BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC y sulfo-SMPB y SVSB (sucinimidil-(4-vinilsulfona)benzoata) que está comercializado (por ejemplo, por Pierce Biotechnology, Inc., Rockford, IL., EE.UU; véanse páginas 467-498, 2003-2004 *Applications Handbook and Catalog.*).

[0466]. Los inmunconjugados que comprenden un anticuerpo y un agente citotóxico también pueden obtenerse utilizando una variedad de agentes de unión a proteína bifuncionales como por ejemplo

N-succinimidil-3-(2-piridilditio) propionato (SPDP), succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato (SMCC), iminotiolano (IT), derivados bifuncionales de imidoésteres (como el dimetiladipimidato HCl), ésteres activos (por ejemplo, disuccinimidil suberato), aldehídos (por ejemplo, glutaraldehídos), compuestos bis-azido (como bis (p-azidobenzoil) hexanodiamina), derivados de bis-diazonio (como bis-(p-diazoniobenzoil)-etilenodiamina), diisocianatos (por ejemplo, 2,6-diisocianato de tolueno) y compuestos bis-activos de fluoruro (como 1,5-difluoruro-2,4-dinitrobenceno). Por ejemplo, una inmunotoxina de ricina se puede preparar tal como se describe en Vitetta y col., *Science*, 238:1098 (1987). El ácido triaminopentaacético 1-isotiocianatobencil-3-metildietileno marcado con carbono 14 (MX-DTPA) es un ejemplo de agente quelante para la conjugación de un radionucleótido con el anticuerpo. Véase el documento WO94/11026.

[0467]. Alternativamente, se puede realizar una fusión de proteínas que contengan un anticuerpo y un agente citotóxico, por ej. mediante técnicas recombinantes o síntesis de péptidos. Una molécula de ADN recombinante puede incluir regiones que codifiquen el anticuerpo y las porciones citotóxicas del conjugado, ya sea adyacente a otro o en forma separada por una región que codifica un péptido de enlace, el cual no destruye las propiedades deseadas del conjugado.

[0468]. En otra forma de realización, un anticuerpo puede conjugarse con un "receptor" (como la estreptavidina) y actuar previamente contra el tumor, donde se administra al paciente el conjugado receptor del anticuerpo, luego, se elimina de la circulación el conjugado no unido, utilizando un agente de eliminación, y posteriormente se administra un "ligando" (por ej. la avidina) que se conjuga con un agente citotóxico (por ej. un radionucleótido).

Preparación de conjugados de anticuerpos anti-STEAP-1

[0469]. Los métodos de diseño, selección y preparación de la invención facilitan asimismo anticuerpos anti-STEAP-1 diseñados con cisteína que son reactivos con funcionalidad electrofílica. Estos métodos facilitan además compuestos de anticuerpos conjugados como compuestos conjugados de anticuerpo y fármaco (ADC) con moléculas de fármaco en puntos específicos, diseñados y selectivos. Los residuos de cisteína del reactivo sobre la superficie de un anticuerpo permiten conjugar específicamente una fracción de fármaco a través de un grupo tiol reactivo como maleimida o haloacetilo. La reactividad nucleofílica de la funcionalidad tiol de un residuo de cisteína con un grupo maleimido es aproximadamente 1.000 veces mayor en comparación con cualquier otra funcionalidad aminoácida en una proteína, como por ejemplo el grupo amino de residuos de lisina o el grupo amino N terminal. La funcionalidad tiol específica en los reactivos yodoacetil y maleimido puede reaccionar con grupos amina, pero se requieren tiempos de reacción mayores a pH (>9,0) y más prolongados (Garman, 1997, *Non-Radioactive Labelling: A Practical Approach*, Academic Press, London). La cantidad de tiol libre en una proteína puede calcularse mediante el ensayo de Ellman estándar. La inmunoglobulina M es un ejemplo de pentámero enlazado con disulfuro,

mientras que la inmunoglobulina G es un ejemplo de una proteína con puentes de disulfuro internos que enlazan a las subunidades. En proteínas como éstas, se requiere la reducción de los enlaces de disulfuro con un reactivo como ditiotreitol (DTT) o selenol (Singh y col. (2002) *Anal. Biochem.* 304:147-156) para generar el reactivo libre de tiol. Este enfoque puede dar como resultado la pérdida de la estructura terciaria del anticuerpo y de la especificidad de unión del antígeno.

[0470]. El Ensayo Pheselector (Ensayo ELISA de fagos para la selección de tioles reactivos) permite la detección de grupos de cisteína reactivos en anticuerpos en un formato ELISA en fagos, con lo cual contribuye al diseño de los anticuerpos diseñados con cisteína (WO 2006/034488). El anticuerpo diseñado con cisteína es revestido en las superficies de los pocillos, seguido por la incubación con partículas de fago, la adición de anticuerpo secundario etiquetado HRP y la detección de la absorbancia. Las proteínas mutantes que se expresan en el fago pueden analizarse mediante un método rápido, sólido y de alto rendimiento. Pueden generarse bibliotecas de anticuerpos diseñados con cisteína y someterlos a selección de unión utilizando el mismo enfoque para identificar adecuadamente los puntos reactivos de incorporación de cisteína libre a partir de bibliotecas aleatorias de proteína-fago de anticuerpos u otras proteínas. Esta técnica incluye la reacción de las proteínas mutantes con cisteína expresadas en el fago con un reactivo de afinidad o un grupo de indicadores que también es tiol reactivo.

[0471]. El ensayo PHESELECTOR permite analizar los grupos tiol específicos presentes en los anticuerpos. La identificación mediante este método de la variante A121C es ejemplar. Es posible buscar eficazmente la totalidad de la molécula Fab para identificar más variantes ThioFab con grupos tiol específicos. Para identificar y cuantificar la accesibilidad del disolvente a los residuos de aminóacido presentes en un polipéptido se emplea una accesibilidad superficial fraccional indicativa. La accesibilidad superficial puede expresarse como la zona de superficie ($Å^2$) que puede contactarse mediante una molécula de disolvente, p.ej. agua. El espacio ocupado por el agua es aproximadamente igual al de una esfera con un radio de 1,4 Å. Puede disponerse del software gratuitamente o bajo licencia (Oficina para CCP4, Daresbury Laboratory, Warrington, WA4 4AD, United Kingdom, Fax: (+44) 1925 603825 o por Internet: www.ccp4.ac.uk/dist/html/INDEX.html) igual que el Paquete Integrado CCP4 de programas de cristalografía que emplea algoritmos para calcular la accesibilidad superficial de cada aminoácido de una proteína con coordenadas derivadas de cristalografía de rayos X conocidas ("El Paquete Integrado CCP4": Programas para cristalografía de proteínas" (1994) *Acta. Cryst.* D50:760-763). Dos módulos de software ejemplares que realizan cálculos de accesibilidad superficial son "AREAIMOL" y "SURFACE", basados en los algoritmos de B.Lee y F.M.Richards (1971) *J.Mol.Biol.* 55:379-400. AREAIMOL define la superficie accesible del disolvente de una proteína como el locus del centro de una esfera sonda (que representa una molécula de disolvente) mientras pasa por encima de la superficie de Van der Waals de la proteína. AREAIMOL calcula la zona superficial accesible del disolvente generando puntos superficiales en una esfera extendida sobre cada átomo (a una distancia del centro del átomo igual a la suma de los

radios del átomo y de la sonda) y eliminando aquellos que se encuentran dentro de las esferas equivalentes asociadas a los átomos adyacentes. AREAIMOL encuentra la zona de átomos accesible al disolvente en un archivo coordinado PDB y resume la zona accesible por residuo, por cadena y para la totalidad de la molécula. Las zonas accesibles (o diferencias de zona) para átomos individuales pueden escribirse en un archivo de salida pseudo-PDB. AREAIMOL atribuye un radio único para cada elemento y sólo reconoce un número limitado de elementos diferentes.

[0472]. AREAIMOL y SURFACE informan sobre las accesibilidades absolutas, es decir el número de Angstroms cuadrados (Å). La accesibilidad superficial fraccional se calcula mediante referencia a un estado estándar aplicable a un aminoácido dentro de un polipéptido. El estado de referencia es un tripéptido Gli-X-Gli, donde X es el aminoácido de interés y el estado de referencia debería ser una conformación 'extendida', es decir similar a la de las hebras beta. La conformación extendida maximiza la accesibilidad de X. La zona accesible calculada se divide por la zona accesible en un estado de referencia de un tripéptido Gly-X-Gly e indica el cociente, que es la accesibilidad fraccional. El porcentaje de accesibilidad es la accesibilidad fraccional multiplicada por 100. Otro algoritmo ejemplar para el cálculo de la accesibilidad se basa en el módulo SOLV del programa xsae (Broger, C., F. Hoffman-LaRoche, Basel) que calcula la accesibilidad fraccional de un residuo de aminoácido a una esfera de agua basándose en las coordenadas de rayos X del polipéptido. La accesibilidad superficial fraccional para cada aminoácido en un anticuerpo puede calcularse usando la información disponible sobre la estructura del cristal (Eigenbron y col. (1993) *J Mol Biol.* 229:969-995).

[0473]. El ADN que codifica los anticuerpos diseñados con cisteína se aísla fácilmente y se secuencia utilizando procedimientos convencionales (por ejemplo, usando sondas de oligonucleótidos capaces de unirse específicamente a los genes que codifican las cadenas pesadas y ligeras de los anticuerpos murinos). Las células de hibridoma sirven como fuente de este ADN. Una vez aislado, el ADN puede colocarse en vectores de expresión, que después se transfectan a células hospederas, como por ejemplo las de *E. coli*, las COS de simios, las de ovario de hámster chino (CHO) o a células hospederas de otros mamíferos, como las células del mieloma (5807715 de EE.UU.; 2005/0048572 de EE.UU.; 2004/0229310 de EE.UU.), que no produzcan la proteína del anticuerpo de alguna otra manera, a fin de obtener la síntesis de anticuerpos monoclonales en las células hospederas recombinantes.

[0474]. Después del diseño y la selección, los anticuerpos diseñados con cisteína, p.ej. ThioFabs, pueden producirse con los residuos de cisteína independientes diseñados altamente reactivos mediante: (i) expresión en un sistema bacteriano, p.ej. *E. coli*, (Skerra y col. (1993) *Curr. Opinion in Immunol.* 5:256-262; Plückthun (1992) *Immunol. Revs.* 130:151-188) o un sistema de cultivo celular en mamíferos (WO 01/00245), p. ej. células de ovario de hamster chino (CHO); y (ii) purificación empleando

las técnicas comunes de purificación de proteínas (Lowman y col. (1991) *J. Biol. Chem.* 266(17):10982-10988).

[0475]. Los grupos tiol Cis diseñados reaccionan con los reactivos ligando electrofílicos y los ligando-fármaco intermedios para formar conjugados de fármaco y anticuerpo diseñados con cisteína y otros anticuerpo marcados diseñados con cisteína. Los residuos de cisteína de los anticuerpos diseñados con cisteína, presentes en los anticuerpos progenitores, que están apareados y forman enlaces disulfuro intercadena e intracadena no tienen ningún grupo tiol específico (a menos que sean tratados con un agente reductor) y no reaccionan con los reactivos ligando electrofílicos o los ligando-fármaco intermedios. Los residuos de cisteína recientemente diseñados pueden permanecer independientes y son capaces de reacccionar, es decir de conjugarse, con un reactivo ligando electrofílico o un ligando-fármaco intermedio, como por ejemplo un fármaco-maleimida. Los ligando-fármaco ejemplares intermedios incluyen: MC-MMAE, MC-MMAF, MC-vc-PAB-MMAE y MC-vc-PAB-MMAF. Las posiciones estructurales de los residuos de cisteína diseñados de las cadenas pesada y ligera se numeran de acuerdo con un sistema de numeración secuencial. Este sistema de numeración secuencial guarda correlación con el sistema de numeración de Kabat (Kabat y col., (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) que comienza en el extremo terminal N y se diferencia del esquema de numeración de Kabat (hilera inferior) en las intercalaciones registradas como a, b y c. Con la utilización del sistema de numeración de Kabat, la secuencia lineal real de aminoácido puede contener menos aminoácidos o aminoácidos adicionales que corresponden a un acortamiento o a una intercalación de un FR o CDR del dominio de la variable. Los lugares variables de la cadena pesada diseñada con cisteína son identificados mediante los esquemas de numeración secuencial y de Kabat.

[0476]. En una forma de realización, el anticuerpo anti-STEAP-1 diseñado con cisteína se prepara mediante un proceso que comprende:

(a) la sustitución de uno o más residuos de aminoácidos de un anticuerpo anti-STEAP-1 progenitor por cisteína; y

(b) la determinación de la reactividad del tiol del anticuerpo anti-STEAP-1 diseñado con cisteína mediante la reacción del anticuerpo diseñado con cisteína con el reactivo tiol-específico.

[0477]. El anticuerpo diseñado con cisteína puede ser más reactivo que el anticuerpo progenitor con el reactivo tiol-específico.

[0478]. Los residuos de aminoácido de cisteína libre pueden localizarse en las cadenas pesadas o ligeras, o en los dominios constantes o variables. Los fragmentos de anticuerpos, p.ej. Fab, también pueden diseñarse con uno o más aminoácidos de cisteína que sustituyen a los aminoácidos del fragmento de anticuerpo para formar fragmentos de anticuerpo diseñados con cisteína.

[0479]. Otra realización de la invención ofrece un método para la preparación (producción) de un anticuerpo anti-STEAP-1 diseñado con cisteína, que comprende:

(a) la introducción de uno o más aminoácidos de cisteína en un anticuerpo anti-STEAP-1 progenitor con el objeto de generar el anticuerpo anti-STEAP-1 diseñado con cisteína; y

(b) la determinación de la reactividad del reactivo tiol-específico diseñado con cisteína con un reactivo tiol-específico;

donde el anticuerpo diseñado con cisteína es más reactivo que el anticuerpo progenitor con el reactivo tiol-específico.

[0480]. El Paso (a) del método de preparación de un anticuerpo diseñado con cisteína puede comprender:

(i) la mutagenización de una secuencia de ácido nucleico que codifica al anticuerpo diseñado con cisteína;

(ii) la expresión del anticuerpo diseñado con cisteína; y

(iii) el aislamiento y la purificación del anticuerpo diseñado con cisteína.

[0481]. El Paso (b) del método de preparación de un anticuerpo diseñado con cisteína puede comprender la expresión del anticuerpo diseñado con cisteína en una partícula viral seleccionada a partir de un fago o de una partícula fagémida.

[0482]. El Paso (b) del método de preparación de un anticuerpo diseñado con cisteína puede comprender:

(i) la reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad tiol específico para generar un anticuerpo diseñado con cisteína de afinidad marcada; y

(ii) la medición de la unión del anticuerpo diseñado con cisteína de afinidad marcada a un medio de captura.

[0483]. Otra realización de la invención es un método de análisis de los anticuerpos diseñados con cisteína con aminoácidos de cisteína independientes altamente reactivos para reactividad del tiol que comprende:

(a) la introducción de uno o más aminoácidos en un anticuerpo progenitor con el objeto de generar un anticuerpo diseñado con cisteína;

(b) la reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad tiol específico para generar un anticuerpo diseñado con cisteína de afinidad marcada; y

(c) la medición de la unión del anticuerpo diseñado con cisteína de afinidad marcada a un medio de captura; y

(d) la determinación de la reactividad del tiol del anticuerpo diseñado con cisteína con un reactivo tiol-específico.

[0484]. El Paso (a) del método de análisis de los anticuerpos diseñados con cisteína puede comprender:

(i) la mutagenización de una secuencia de ácido nucleico que codifica al anticuerpo diseñado con cisteína;

(ii) la expresión del anticuerpo diseñado con cisteína; y

(iii) el aislamiento y la purificación del anticuerpo diseñado con cisteína.

[0485]. El Paso (b) del método de análisis de anticuerpos diseñados con cisteína puede comprender la expresión del anticuerpo diseñado con cisteína en una partícula viral seleccionada a partir de un fago o de una partícula fagémida.

[0486]. El Paso (b) del método de análisis de los anticuerpos diseñados con cisteína también puede comprender:

(i) la reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad tiol específico para generar un anticuerpo diseñado con cisteína de afinidad marcada; y

(ii) la medición de la unión del anticuerpo diseñado con cisteína de afinidad marcada a un medio de captura.

Anticuerpos anti-STEAP-1 marcados diseñados con cisteína

[0487]. Los anticuerpos anti-STEAP-1 diseñados con cisteína pueden ubicarse específicamente y acoplarse eficazmente con un reactivo tiol-específico. El reactivo tiol-específico puede ser un reactivo ligando multifuncional, un reactivo de captura marcado (p.ej. un reactivo de enlace con biotina), una marca de detección (p.ej. un reactivo fluoróforo), un reactivo de inmovilización de fase sólida (p.ej. SEPHAROSE™, poliestireno o vidrio) o un ligando-fármaco intermedio. Un ejemplo de un reactivo tiol-específico es N-etil maleimida (NEM). En una realización ejemplar, la reacción de un ThioFab con un reactivo de enlace con biotina suministra un ThioFab biotinilado mediante el cual puede detectarse y medirse la presencia y la reactividad del residuo de cisteína diseñado. La reacción de un ThioFab con un reactivo ligando multifuncional suministra un ThioFab con un ligando funcionalizado que además puede hacerse reaccionar con un reactivo de una reacción del fármaco u otro marcador. La reacción de un ThioFab con un ligando-fármaco intermedio suministra un conjugado de fármaco y ThioFab.

[0488]. En general, los métodos ejemplares descritos en esta memoria pueden aplicarse a la identificación y producción de anticuerpos, y más comúnmente a otras proteínas a través de la aplicación de los pasos de diseño y análisis aquí descritos.

[0489]. Dicho enfoque puede aplicarse a la conjugación de otros reactivos tiol-específicos en los que el grupo reactivo es, por ejemplo, un maleimido, una iodoacetamida, un piridil disulfuro u otra molécula de conjugación tiol reactiva (Haugland, 2003, *Molecular Probes Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc.; Brinkley, 1992, *Bioconjugate Chem.* 3:2; Garman, 1997, *Non-Radioactive Labelling: A Practical Approach,* Academic Press, London; Means (1990) *Bioconjugate Chem.* 1:2; Hermanson, G. en *Bioconjugate Techniques* (1996) Academic Press, San Diego, págs. 40-55, 643-671). El reactivo tiol-específico puede ser una fracción de fármaco, un fluoróforo como por ejemplo un tinte fluorescente como la fluoresceína o rodamina, un agente quelante para un diagnóstico por imágenes o un metal radioterapéutico, una marca peptidil o no peptidil o una marca de detección, o un agente modificador de la eliminación, como por ejemplo diversos isómeros del polietilenglicol, un péptido que se une a un tercer componente, u otro carbohidrato o agente lipofílico.

Usos de anticuerpos anti-STEAP-1 diseñados con cisteína

[0490]. Los anticuerpos anti-STEAP-1 diseñados con cisteína, y los conjugados de los mismos, pueden emplearse como agentes terapéuticos y/o de diagnóstico. La presente invención ofrece además métodos para prevenir, controlar, trata o mejorar uno o más síntomas asociados a un trastorno relacionado con STEAP-1. En particular, la presente invención ofrece métodos para prevenir, controlar, tratar o mejorar uno o más síntomas asociados al trastorno de proliferación celular, como un cáncer, p.ej., cáncer de próstata, de pulmón, de colon, de vejiga y de ovario, y el sarcoma de Ewing. La presente invención ofrece además métodos para el diagnóstico de un trastorno relacionado con STEAP-1 o la predisposición a desarrollar dicho trastorno, así como métodos para identificar anticuerpos, y fragmentos de anticuerpos de unión a antígeno, que preferentemente se unen a los polipéptidos STEAP-1 asociados a las células.

[0491]. Otra forma de realización de la presente invención se orienta hacia el uso de un anticuerpo anti-STEAP-1 diseñado con cisteína para la preparación de un medicamento útil para el tratamiento de un estado receptivo a un trastorno relacionado con STEAP-1.

Preparación de conjugados de fármaco con anticuerpos anti-STEAP-1 diseñados con cisteína

[0492]. Los ADC de la formula I se pueden preparar por varios medios, empleando reacciones químicas orgánicas, condiciones y reactivos conocidos por los expertos, como: (1) una reacción de un grupo de cisteínas de un anticuerpo diseñado con cisteína con un agente enlazador, para formar un enlazador a anticuerpo intermedio Ab-L, mediante un enlace covalente, seguida de la reacción con una fracción del fármaco D activado; y (2) una reacción de un grupo nucleofílico de una fracción del fármaco con un reactivo enlazador, para formar un enlazador a fármacos intermedio D-L, mediante un enlace covalente, seguido de la reacción con el grupo de cisteínas de un anticuerpo diseñado con cisteína. Los métodos de conjugación (1) y (2) pueden emplearse con una variedad de anticuerpos diseñados con

cisteína, fracciones de fármacos y enlazadores para preparar conjugados de anticuerpos con fármacos de Fórmula I.

[0493]. Los grupos tiol de cisteína del anticuerpo son nucleofílicos y son capaces de reaccionar para formar enlaces covalentes con grupos electrofílicos en los reactivos enlazadores y los enlazadores a fármacos intermedios, que incluyen: (i) ésteres activos como los ésteres NHS, los ésteres HOBt, haloformos y haluros de ácido; (ii) haluros de alquilo y bencilo como las haloacetamidas; (iii) aldehídos, cetonas, carboxilo y grupos maleimidos, y (iv) disulfuros, incluyendo piridil disulfuros, mediante un intercambio de sulfuros. Los grupos nucleofílicos en una fracción del fármaco incluyen los siguientes compuestos: los grupos amino, tiol, hidroxilo, hidracida, oxima, hidracina, tiosemicarbazona, carboxilato de hidracina y aril-hidracida, capaces de reaccionar para formar enlaces covalentes con grupos electrofílicos en las fracciones enlazadoras y reactivos enlazadores.

[0494]. Los anticuerpos diseñados con cisteína pueden convertirse en reactivos para conjugarse con reactivos enlazadores mediante el tratamiento con un agente reductor como DTT (reactivo de Cleland, ditiotreitol) o TCEP (tris(2-carboxietil)fosfino hidrocloruro; Getz y col., (1999) *Anal. Biochem.* Vol. 273:73-80; Soltec Ventures, Beverly, MA), seguido de reoxidación para reformar los enlaces disulfuros de la intercadena e intracadena (Ejemplo x). Por ejemplo, los anticuerpos monoclonales diseñados con cisteína de longitud completa (ThioMab) expresados en las células CHO se reducen con un exceso de TCEP de aproximadamente 50 veces durante 3 horas a 37 °C para reducir los enlaces disulfuros en los aductos de cisteínas, que pueden formarse entre los residuos de cisteína introducidos recientemente y la cisteína presente en el medio de cultivo. El ThioMab reducido se diluye y se carga en la columna HiTrap S en acetato sódico 10 mM, pH 5, y se eluye con PBS que contiene cloruro de sodio 0,3 M. Los enlaces disulfuros se restablecieron entre los residuos de cisteína presentes en el Mab parental con sulfato de cobre ($CuSO_4$) acuoso diluído (200 nM) a temperatura ambiente, durante toda la noche. Alternativamente, el ácido dehidroascórbico (DHAA) es un oxidante eficaz para restablecer los grupos disulfuros de la intracadena del anticuerpo diseñado con cisteína después de reducir la escisión de aductos de cisteína. Se pueden utilizar otros oxidantes, es decir agentes oxidantes, y condiciones oxidantes, que son bien conocidos en la materia. La oxidación al aire del ambiente también es eficaz. Este paso de reoxidación leve y parcial forma eficazmente disulfuros de intracadenas con una alta fidelidad y conserva los grupos tiol de los residuos de cisteína introducidos recientemente. Se agregó y mezcló un exceso de aproximadamente 10 veces del enlazador a fármaco intermedio, como por ejemplo, MC-vc-PAB-MMAE, y se lo dejó reposar durante aproximadamente una hora a temperatura ambiente para lograr una conjugación y formar el conjugado de anticuerpo con fármacos. La mezcla de conjugación se filtró en gel y se cargó y eludió a través de una columna HiTrap S para eliminar el exceso del enlazador a fármaco intermedio y otras impurezas.

[0495]. La Figura 16 muestra el proceso general para preparar un anticuerpo diseñado con cisteína expresado desde el cultivo celular para la conjugación. Cuando el medio de cultivo celular contiene cisteína, los aductos de disulfuros pueden formarse entre los aminoácidos con cisteína introducidos recientemente y las cisteínas del medio. Estos aductos de cisteínas, que se muestran como un círculo en el ThioMab ejemplar (izquierda) en la Figura 12, deben reducirse para generar anticuerpos reactivos diseñados con cisteína para la conjugación. Los aductos de cisteínas, supuestamente junto con varios enlaces disulfuros de intercadenas, se escinden de manera reduccionista para obtener una forma reducida del anticuerpo con agentes reductores tal como TCEP. Los enlaces disulfuros de intercadenas entre residuos de cisteína emparejados se reforman bajo condiciones de oxidación parcial con sulfato de cobre, DHAA o con la exposición al oxígeno del ambiente. Los residuos de cisteína introducidos recientemente, diseñados e independientes permanecen disponibles para la reacción con los reactivos enlazadores o los enlazadores a fármacos intermedios para formar los conjugados de anticuerpos de la invención. Los ThioMab expresados en las líneas celulares de mamíferos dan lugar a cis conjugados externamente aducidos a un cis diseñado mediante una formación de unión –S-S-. Por lo tanto, los ThioMab purificados se tratan con los procedimientos de reducción y reoxidación, tal como se describen en el Ejemplo x, para producir ThioMab reactivos. Estos ThioMab se utilizan para conjugarse con fármacos citotóxicos que contienen maleimidos, con fluoroforos y con otras marcas.

[0496]. La Figura 15 muestra realizaciones de los conjugados de anticuerpos con fármacos (ADC) anti-STEAP-1 diseñado con cisteína, donde la porción del fármaco auristatina se une a un grupo diseñado con cisteína en: la cadena ligera (LC-ADC); la cadena pesada (HC-ADC); y en la región Fc (Fc-ADC).

Formulaciones farmacéuticas

Administración de los conjugados de anticuerpos con fármacos

[0497]. Los conjugados de anticuerpos con fármacos (ADC), de la invención pueden administrarse mediante cualquier vía adecuada según la afección que deba tratarse. Por lo general, los ADC se administrarán parenteralmente, es decir, por infusión, por vía subcutánea, intramuscular, intravenosa, intradérmica, intratecal o epidural.

[0498]. Para el tratamiento de cánceres de, por ejemplo, próstata, pulmón y/o colon, en una forma de realización, el conjugado de anticuerpo con fármacos se administra por infusión intravenosa. La dosis que se administra por infusión varía aproximadamente entre 1 $\mu g/m^2$ y 10.000 $\mu g/m^2$ por dosis, por lo general, una dosis semanal de un total de una, dos, tres o cuatro dosis. Alternativamente, el rango de dosis es aproximadamente de entre 1 $\mu g/m^2$ y aproximadamente 1000 $\mu g/m^2$, aproximadamente 1 $\mu g/m^2$ y aproximadamente 800 $\mu g/m^2$, aproximadamente 1 $\mu g/m^2$ y aproximadamente 600 $\mu g/m^2$, aproximadamente 1 $\mu g/m^2$ y aproximadamente 400 $\mu g/m^2$, aproximadamente 10 $\mu g/m^2$ y aproximadamente 500 $\mu g/m^2$,

aproximadamente 10 $\mu g/m^2$ y aproximadamente 300 $\mu g/m^2$, aproximadamente 10 $\mu g/m^2$ y aproximadamente 200 $\mu g/m^2$, y aproximadamente 1 $\mu g/m^2$ y 200 aproximadamente $\mu g/m^2$. La dosis puede administrarse una vez al día, una vez a la semana, varias veces a la semana, pero menos de una vez al día; varias veces al mes, pero menos de una vez al día; varias veces al mes, pero menos de una vez por semana; una vez al mes o intermitentemente para aliviar los síntomas de la enfermedad. La administración puede continuar en cualquiera de los intervalos mencionados anteriormente hasta lograr la remisión del tumor o los síntomas del linfoma de la leucemia que se trata. La administración puede continuar después de lograr la remisión o el alivio de los síntomas, siempre que dicha remisión o alivio se mantengan debido a la administración continua.

[0499]. La invención también proporciona un método de tratamiento de un cancer de próstata, pulmón y/o colon, y/o una metástasis de dicho cáncer, que comprende la administración al paciente que padezca de cáncer de próstata, pulmón o colon, de una cantidad terapéuticamente eficaz de un anticuerpo humanizado 120v.24 de cualquiera de las formas de realización precedente dicho anticuerpo no está conjugado con una molécula citotóxica ni con una molécula detectable. Por lo general, el anticuerpo se administrará con una dosis que varía entre 1 $\mu g/m^2$ y aproximadamente 1000 mg/m^2.

[0500]. La invención también ofrece un método para el tratamiento de un cáncer de próstata, pulmón y/o colon, y/o una metástasis de dicho cáncer, que comprende la administración al paciente que sufre un cáncer de próstata, pulmón o colon de una cantidad terapéuticamente eficaz de un anticuerpo 120v.24 humanizado de cualquiera de una de las realizaciones anteriores, en la cual el anticuerpo está conjugado con una molécula citotóxica o detectable. Por lo general, el anticuerpo se administrará en una dosis que varía de aproximadamente 1 $\mu g/m^2$ a aproximadamente 1.000 mg/m^2.

[0501]. En un aspecto, la invención además proporciona formulaciones farmacéuticas que comprenden al menos un anticuerpo anti-STEAP-1 de la invención, al menos un inmunoconjugado de éste o al menos un conjugado de anticuerpo anti-STEAP-1 con fármacos de la invención, o todos los anteriores. En algunas formas de realización, una formulación farmacéutica comprende 1) un anticuerpo anti-STEAP-1, un conjugado de anticuerpo anti-STEAP-1 con fármacos o un inmunoconjugado de éste, o todos los anteriores, y 2) un vehículo farmacéuticamente aceptable. En algunas formas de realización, una formulación farmacéutica comprende 1) un anticuerpo anti-STEAP-1 o un inmunoconjugado de éste, o ambos, y opcionalmente 2) al menos un agente terapéutico adicional.

[0502]. Las formulaciones farmacéuticas que comprenden un anticuerpo, inmunoconjugado o un conjugado de anticuerpo anti-STEAP-1 con fármacos de la invención se preparan para su almacenamiento mezclando el anticuerpo o el conjugado de anticuerpo con fármacos que tenga el grado de pureza deseado con vehículos, excipientes o estabilizadores opcionales que sean fisiológicamente aceptables (*Remington's Pharmaceutical Sciences* 16.ª edición, Osol, A. Ed. (1980)), en forma de

formulaciones acuosas, liofilizadas u otras formulaciones liofilizadas. Los vehículos, excipientes o estabilizadores aceptables no son tóxicos para los receptores en todas las dosis y concentraciones empleadas, e incluyen soluciones tamponadas tales como fosfato, citrato, histidina y otros ácidos orgánicos; antioxidantes que incluyen ácido ascórbico y metionina; conservantes (como por ejemplo, cloruro amonio de octadecildimetilbencilo; cloruro de hexametonio; cloruro de benzalconio, cloruro de benzetonio; alcohol bencilo, fenol o butilo benzil alcohol; alquilo parabeno como el metal o propel parabeno; catecol; resorcinol; ciclohexanol; 3-pentanol; y m-cresol); bajo peso molecular (menos de 10 residuos) polipéptidos; proteínas, como cera albúmina, gelatina, o inmunoglobulinas; polímetros hidroilicos como la polivinilpirrolidone; aminoácidos como glicerina, glutamina, asparagina, histidina, arginina, o lisina; monosacaridos, disacáridos y otros carbohidratos que incluyen glucosa, manosa, o dextrinas; agentes quelantes como la EDTA; azúcares como sucrosa, manitol, trehalosa o sorbitol; contra-iones de formación salina como el sodio; complejos metálicos (por ejemplo, complejos Zn-proteína); y/o tensoactivos no surfactantes como TWEEN™, PLURONICS™ o polietilenglicol (PEG). Las formulaciones farmacéuticas para administración *in vivo* deben ser, por lo general, estériles. Esto se logra mediante la filtración a través de membranas de filtración estériles.

[0503]. Los principios activos también se pueden atrapar en microcápsulas preparadas, por ejemplo, mediante técnicas de coacervación o polimerización interfacial, por ejemplo, hidroximetilcelulosa o microcápsulas de gelatina y poli-(metilmetacrilato), respectivamente, en sistemas de administración de fármacos coloidales (por ejemplo, liposomas, microesferas de albúmina, microemulsiones, nanopartículas y nanocápsulas), o en macroemulsiones. Dichas técnicas se dan a conocer en *Remington's Pharmaceutical Sciences*, 16.ª edición, Oslo, A., Ed. (1980).

[0504]. Se pueden elaborar preparaciones de liberación controlada. Ejemplos adecuados de preparaciones de liberación controlada incluyen matrices semipermeables de polímeros hidrófobos sólidos que contienen el anticuerpo o inmunoconjugado de la invención, cuyas matrices están en forma de elementos moldeados, por ejemplo, películas o microcápsulas. Ejemplos de matrices de liberación controlada incluyen poliésteres, hidrogeles (por ejemplo, poli-(2-hidroxietil-metacrilato), o poli-(vinilalcohol)), poliláctidos (patente de EE.UU. N.º 3.773.919), copolímeros de ácido L-glutámico e γ etil-L-glutamato, acetato de etilenvinilo no degradable, copolímeros degradables de ácido glicólico-ácido láctico como por ejemplo LUPRON DEPOT™ (microesferas inyectables compuestas por copolímeros de ácido glicólico-ácido láctico y acetato de leuprolida) y ácido poli-D-(-)-3-hidroxibutírico. Mientras que los polímeros como el acetato de etilenvinilo y el ácido glicólico-ácido láctico permiten la liberación de moléculas por un período de 100 días, ciertos hidrogeles liberan proteínas durante períodos de tiempo menores. Cuando los anticuerpos o inmunoconjugados encapsulados permanecen en el organismo durante un largo periodo de tiempo, pueden desnaturalizarse o agregarse como resultado de la exposición a la

humedad a 37 °C, resultando en una pérdida de la actividad biológica y en posibles cambios en la inmunogenicidad. Es posible concebir estrategias racionales para su estabilización dependiendo del mecanismo que intervenga. Por ejemplo, si se descubre que el mecanismo de agregación es de formación de uniones intermoleculares S-S mediante el intercambio tio-disulfuro, se puede lograr la estabilización mediante la modificación de los residuos sulfhidrilos, liofilizando soluciones ácidas, controlando la humedad, utilizando aditivos adecuados y desarrollando composiciones de matrices de polímeros especificas.

Tratamientos de conjugados de anticuerpos con fármacos

[0505]. Se contempla que el conjugado de anticuerpo con fármacos (ADC) de la presente invención puede utilizarse para tratar diversas enfermedades o trastornos, como por ejemplo, aquellos caracterizados por la sobreexpresión de un antígeno tumoral. Las afecciones o trastornos hiperproliferativos ejemplares incluyen tumores benignos y malignos, leucemia y neoplasias linfoides. Otros incluyen trastornos neuronales, gliales, astrocitales, hipotalámicos, glandulares, macrofágicos, epitetales, estromales, blastocélicos, inflamatorios, angiogénicos e inmunológicos, incluyendo los autoinmunes. Otros incluyen cánceres de próstata, pulmón y colon.

[0506]. Los componentes del ADC que se identifican en los modelos animales y ensayos con células pueden evaluarse en estudios clínicos de humanos y primates mayores con tumores. Se pueden diseñar estudios clínicos con humanos para evaluar la eficacia del anticuerpo monoclonal anti-STEAP-1 o el inmunoconjugado de la invención en pacientes con trastornos proliferativos de células de próstata, pulmón o colon, incluyendo, entre otros, cánceres de próstata, pulmón y colon y metástasis de dichos cánceres. El estudio clínico se puede diseñar para evaluar la eficacia de un ADC en combinación con regímenes terapéuticos conocidos, tales como la radiación o la quimioterapia, o ambas, que implican agentes quimioterapéuticos o citotóxicos conocidos.

[0507]. El cáncer puede comprender células que expresan STEAP-1 para que un ADC de la presente invención sea capaz de unirse a las células cancerosas. Existen diversos ensayos diagnósticos/prognósticos para determinar la expresión de STEAP-1 en el cáncer. En una forma de realización, la sobreexpresión de STEAP-1 puede analizarse mediante un ensayo inmunohistoquímico (IHC). Las secciones de tejido de una biopsia tumoral embebidas en parafina pueden someterse a un ensayo IHC y evaluarse según el criterio de intensidad de tinción proteínica de STEAP-1 con respecto al grado de tinción y la proporción de las células tumorales examinadas.

[0508]. Para la prevención o tratamiento de la enfermedad, la dosis adecuada del ADC dependerá del tipo de enfermedad que se tratará, tal como se define anteriormente, la gravedad y el curso de la enfermedad, si la molécula se administra para fines preventivos o terapéuticos, la terapia anterior, la historia clínica del paciente y la respuesta al anticuerpo, y la discreción del médico tratante. La molécula se

administra adecuadamente al paciente una vez o en una serie de tratamientos. Dependiendo del tipo y la gravedad de la enfermedad, una dosis inicial candidata para la administración en un paciente es de aproximadamente 1 μg/kg a 15 mg/kg (por ejemplo, 0,1-20 mg/kg) de la molécula, ya sea, por ejemplo, mediante una o más administraciones separadas o mediante una infusión continua. Una dosis diaria típica podría oscilar entre aproximadamente 1 μg/kg y 100 mg/kg o más, dependiendo de los factores mencionados anteriormente. Una dosis ejemplar de ADC que se administrará al paciente varía entre 0,1 y aproximadamente 10 mg/kg del peso del paciente.

[0509]. Para administraciones repetidas durante varios días o más, dependiendo de la enfermedad, el tratamiento se mantiene hasta que se produzca la supresión deseada de los síntomas de la misma. Un ejemplo de la pauta posológica es la administración de una dosis inicial de carga de aproximadamente 4 mg/kg, seguida de una dosis de mantenimiento semanal de aproximadamente 2 mg/kg de un anticuerpo anti-STEAP-1. Otras pautas posológicas pueden ser útiles. El progreso de este tratamiento se controla fácilmente mediante técnicas y ensayos convencionales.

Terapia combinada

[0510]. Un conjugado de anticuerpo con fármacos (ADC) de la invención puede combinarse en una formulación farmacéutica combinada o una pauta posológica como una terapia combinada, con un componente adicional, por lo menos, con propiedades antineoplásicas. Por lo menos, un compuesto adicional de la formulación farmacéutica combinada o la pauta posológica tiene preferentemente actividades complementarias al ADC de la combinación para que no se afecten de manera adversa entre sí.

[0511]. Un compuesto adicional, por lo menos, puede ser un agente quimioterapéutico, un agente citotóxico, una citoquina, un agente inhibidor del crecimiento, un agente antihormonal o un cardioprotector, o todos los anteriores. Dichas moléculas están presentes en combinación en cantidades que son eficaces para el fin que se pretende. Una composición farmacéutica que contiene un ADC de la invención también puede tener una cantidad terapéuticamete eficaz de un agente quimioterapéutico tal como un inhibidor que forma tubulina, un inhibidor de la topoisomerasa o un aglutinante a ADN.

[0512]. En un aspecto, el primer compuesto es un ADC anti-STEAP-1 de la invención y por lo menos un compuesto adicional es un anticuerpo terapéutico aparte de un anti-STEAP-1 (anticuerpo desnudo o un ADC). En una forma de realización, por lo menos, el compuesto adicional es el anticuerpo anti-PSCA. En una forma de realización, por lo menos un compuesto adicional es el anticuerpo anti-HER2, trastuzumab (por ejemplo, Herceptin®, Genentech, Inc., South San Francisco, CA). En una forma de realización, por lo menos un compuesto adicional es el anticuerpo anti-HER2, pertuzumab (Omnitarg™, Genentech, Inc., South San Francisco, CA, ver EE.UU. 6949245). En una realización, al menos un compuesto adicional es un anticuerpo anti-VEGF (p.ej. Avastin®, Genentech, Inc.). En cada caso, al menos un compuesto es un anticuerpo desnudo o un ADC. En una realización, al menos un compuesto adicional es

un anticuerpo (un anticuerpo desnudo o un ADC), y el anticuerpo adicional es un anticuerpo segundo, tercero, cuarto, quinto, sexto o más, de manera que una combinación de anticuerpos segundos, terceros, cuartos, quintos, sextos o más (desnudos o como un ADC) resulta eficaz para el tratamiento de un trastorno de proliferación celular en un tejido que expresa STEAP-1.

[0513]. Otros regímenes terapéuticos pueden combinarse con la administración de un agente antineoplásico identificado según esta invención, incluyendo, entre otros, la radioterapia, transplantes de médula ósea o sangre periférica, un agente citotóxico, un agente quimioterapéutico o un agente inhibidor del crecimiento, o todos los anteriores. En una de estas formas de realización, un agente quimioterapéutico es un agente o una combinación de agentes, como por ejemplo, ciclofosfamida, hidroxidaunorubicina, adriamicina, doxorrubicina, vincristina (Oncovin™), prednisolona, CHOP, CVP o COP, o inmunoterapéuticos tales como anti-PSCA, anti-HER2 (por ejemplo, Herceptin®, Omnitarg™) o anti-VEGF (por ejemplo, Avastin®). La terapia combinada puede administrarse como un régimen simultáneo o secuencial. Cuando se administra de manera secuencial, la combinación puede administrarse en dos o más administraciones. La administración combinada incluye la coadministración, utilizando formulaciones separadas o una única formulación farmacéutica, y la administración consecutiva en cualquier orden, en la que preferentemente exista un período en el cuál ambos (o todos los) agentes activos ejerzan simultáneamente sus actividades biológicas.

[0514]. En una forma de realización, el tratamiento con un ADC supone la administración combinada de un agente antineoplásico identificado en la presente memoria descriptiva y uno o más agentes quimioterapéuticos o agentes inhibidores del crecimiento, incluida la coadministración de un cóctel de diferentes agentes quimioterapéuticos. Entre los agentes quimioterapéuticos están los taxanos (como paclitaxel y docetaxel) o antibióticos a base de antraciclina, o ambos. La preparación y programaciones de la dosis de dichos agentes quimioterapéuticos se pueden utilizar de acuerdo con las instrucciones del fabricante o como lo determine empíricamente el médico cualificado. La preparación y programación de la dosis para dicha quimioterapia también se describen en "*Chemotherapy Service*", (1992) Ed., M.C. Perry, Williams y Wilkins, Baltimore, Md.

[0515]. Las dosis adecuadas para cualquiera de los agentes coadministrados antes mencionados son aquellas que se utilizan actualmente y pueden disminuirse debido a la acción combinada (sinergia) del agente identificado recientemente y otros agentes o tratamientos quimioterapéuticos.

[0516]. La terapia combinada puede proporcionar "sinergia" y ser "sinérgico", es decir, el efecto que se logra cuando los ingredientes activos utilizados en conjunto es mayor a la suma de los efectos que se obtienen del uso de los componentes por separado. Se puede obtener un efecto sinérgico cuando los ingredientes activos se: (1) coformulan y administran simultáneamente en una formulación combinada de dosis unitaria; (2) se administran de manera alternada o paralela como formulaciones separadas; o (3)

mediante algún otro régimen. Cuando se administran en una terapia alterna, se puede obtener un efecto sinérgico cuando los compuestos se administran secuencialmente, por ejemplo, mediante diferentes inyecciones en jeringas separadas. En general, durante la terapia alternada, se administra una dosis eficaz de cada ingrediente activo de manera secuencial, es decir, en serie, mientras que en la terapia combinada, las dosis eficaces de dos o más ingredientes activos se administran juntas.

Metabolitos de los conjugados de anticuerpos con fármacos

[0517]. También se encuentran dentro del campo de esta invención los productos metabólicos *in vivo* de los compuestos de los ADC descritos en la presente memoria descriptiva, hasta el punto que estos productos son novedosos e imperceptibles hasta el momento. Dichos productos pueden originarse, por ejemplo, de la oxidación, reducción, hidrólisis, amidación, esterificación, escisión enzimática, y similares, de los compuestos administrados. Por consiguiente, la invención incluye compuestos novedosos e imperceptibles producidos mediante un proceso que comprende establecer un contacto entre un compuesto de esta invención y un mamífero durante un periodo de tiempo suficiente para producir un producto metabólico de dicho compuesto.

[0518]. Por lo general, los productos metabolitos se identifican al preparar un ADC radiomarcado (por ejemplo, ^{14}C o ^{3}H) que se administra parenteralmente a un animal, como por ejemplo, una rata, un ratón, un cobaya, un mono, o a un humano en una dosis detectable (por ejemplo, mayor a 0,5 mg/kg aproximadamente) y permitir el tiempo suficiente para que se produzca el metabolismo (por lo general, aproximadamente entre 30 segundos y 30 horas) y aislar sus productos de conversión de la orina, sangre u otras muestras biológicas. Estos productos se aíslan fácilmente ya que están marcados (otros se aíslan mediante el uso de anticuerpos capaces de unir epítopos que sobreviven en el metabolito). Las estructuras de los metabolitos se determinan de manera convencional, por ejemplo, mediante espectometría de masas (MS), cromatografía líquida/espectometría de masas (LC/MS) o resonancia magnética nuclear (NMR). En general, el análisis de metabolitos se realiza de la misma manera que los estudios convencionales del metabolismo de fármacos que son bien conocidos por los expertos en la materia. Los productos de conversión, siempre que no se encuentren *in vivo*, son útiles en los ensayos de diagnóstico para establecer las dosis terapéuticas de los compuestos de ADC de la invención.

Otros métodos de utilización de anticuerpos anti-STEAP-1 e inmunoconjugados

Métodos diagnósticos y métodos de detección

[0519]. En un aspecto, los anticuerpos anti-STEAP-1 y los inmunoconjugados de la invención son útiles para detectar la presencia de STEAP-1 en una muestra biológica. Por "detectar" se entiende en la presente memoria descriptiva una detección cuantitativa o cualitativa. En ciertas formas de realización, una muestra biológica comprende una célula o un tejido. En ciertas formas de realización, estos tejidos

incluyen tejidos normales o cancerosos, o ambos, que expresan STEAP-1 a niveles más altos que en otros tejidos, por ejemplo, próstata, pulmón o colon.

[0520]. En un aspecto, la invención proporciona un método de detección de la presencia de STEAP-1 en una muestra biológica. En ciertas formas de realización, el método comprende establecer un contacto de la muestra biológica con un anticuerpo anti-STEAP-1 en condiciones que faciliten la unión del anticuerpo anti-STEAP-1 a STEAP-1 y detectar si se ha formado un complejo entre el anticuerpo anti-STEAP-1 y STEAP-1.

[0521]. En un aspecto, la invención proporciona un método para diagnosticar un trastorno asociado a un aumento de la expresión de STEAP-1. En ciertas formas de realización, el método supone poner en contacto una célula de prueba con un anticuerpo anti-STEAP-1; determinar el nivel de expresión (bien cuantitativo o cualitativo) de STEAP-1 por la célula de prueba mediante la detección de la unión del anticuerpo anti-STEAP-1 a STEAP-1; y comparar el nivel de expresión de STEAP-1 por la célula de prueba con el nivel de expresión de STEAP-1 por una célula de control (por ejemplo, una célula normal del mismo origen tisular que la célula de prueba o una célula que exprese STEAP-1 a niveles similares a dicha célula normal), donde un nivel más alto de expresión de STEAP-1 de la célula de prueba con respecto a la célula de control indica la presencia de un trastorno asociado a un aumento de la expresión de STEAP-1. En ciertas formas de realización, la célula de prueba se obtiene a partir de un sujeto que se sospecha que tiene un trastorno asociado a un aumento de la expresión de STEAP-1. En ciertas formas de realización, el trastorno es un trastorno de proliferación celular, como un cáncer o un tumor.

[0522]. Los trastornos proliferativos celulares ejemplares que pueden diagnosticarse mediante un anticuerpo de la invención incluyen cánceres de próstata, pulmón y colon y metástasis de dichos cánceres.

[0523]. En ciertas formas de realización, un método de diagnóstico o detección, como los descritos anteriormente, comprende la detección de la unión de un anticuerpo anti-STEAP-1 a STEAP-1 expresado en la superficie de una célula o en una preparación de membrana obtenida de una célula que exprese STEAP-1 en su superficie. En ciertas formas de realización, el método comprende establecer un contacto de una célula con un anticuerpo anti-STEAP-1 en condiciones que faciliten la unión del anticuerpo anti-STEAP-1 a STEAP-1 y detectar si se ha formado un complejo entre el anticuerpo anti-STEAP-1 y STEAP-1 en la superficie celular. Un ensayo ejemplar para detectar la unión de un anticuerpo anti-STEAP-1 a STEAP-1 que exprese STEAP-1 en la superficie de una célula es un ensayo "FACS".

[0524]. Se pueden utilizar otros métodos para detectar la unión de anticuerpos anti-STEAP-1 a STEAP-1. Estos métodos incluyen ensayos de unión a antígeno que son bien conocidos en la materia, como Western blot, radioinmunoensayos, ELISA (ensayos de inmunoabsorción enzimática),

inmunoensayos tipo "sandwich", ensayos de inmunoprecipitación, inmunoensayos fluorescentes, inmunoensayos de proteína A y ensayos inmunohistoquímicos (IHC).

[0525]. En ciertas formas de realización, los anticuerpos anti-STEAP-1 están marcados. Las marcar incluyen marcas o fracciones que se detectan directamente (como etiquetas fluorescentes, cromofóricas, densas en electrones, quimioluminescentes y radioacivas), así como fracciones, como enzimas o ligandos, que se detectan indirectamente, por ejemplo, mediante una reacción enzimática o interacción molecular. Ejemplos de estos marcadores incluyen, entre otros, los radioisotopos ^{32}P, ^{14}C, ^{125}I, ^{3}H y ^{131}I, fluoroforos como algunos quelantes térreos raros o fluoresceína y sus derivados, rodamina y sus derivados, dansil, umbeliferon, luceriferasas, por ejemplo, luciferasa de la luciérnaga y luciferasa bacteriana (patente de EE.UU. N.° 4.737.456), luciferina, 2,3-dihidroftalazinadionas, peroxidasa de rábano picante (HPR), fosfatasa alcalina, beta-galactosidasa, glucoamilasa, lisozima, oxidasas sacáridas, por ejemplo, glucosa oxidasa, galactosa oxidasa y glucosa-6-fosfato deshidrogenasa, oxidasas heterocíclicas como uricasa y xantina oxidasa, acopladas con una enzima que emplea peróxido de hidrógeno para oxidar un precursor de tinte como HRP, lactoperoxidasa o microperoxidasa, biotina/avidina, marcadores rotatorios, marcadores bacteriófagos, radicales libres estables y similares.

[0526]. En ciertas formas de realización, los anticuerpos anti-STEAP-1 están inmovilizados en una matriz insoluble. La inmovilización supone separar el anticuerpo anti-STEAP-1 de cualquier STEAP-1 que permanezca libre en solución. Tradicionalmente, esto se consigue bien insolubilizando el anticuerpo anti-STEAP-1 antes del procedimiento de ensayo, o bien mediante adsorción en una superficie o matriz insoluble al agua (Bennich y col., patente de EE.UU. No.` 3.720.760), o bien mediante acoplamiento covalente (por ejemplo, usando reticulación con glutaraldehído), o bien mediante insolubilización del anticuerpo anti-STEAP-1 después de la formación de un complejo entre el anticuerpo anti-STEAP-1 y STEAP-1, por ejemplo, mediante inmunoprecipitación.

[0527]. Cualquiera de las formas de realización anteriores para diagnóstico o detección se pueden llevar a cabo utilizando un inmunoconjugado de la invención en lugar de un anticuerpo anti-STEAP-1 o en adición a él.

Métodos terapéuticos

[0528]. Un anticuerpo o inmunoconjugado de la invención se pueden usar, por ejemplo, en métodos terapéuticos *in vitro, ex vivo* e *in vivo*. En un aspecto, la invención proporciona métodos para inhibir el crecimiento o la proliferación celular bien *in vivo* o *in vitro* y dicho método comprende la exposición de una célula a un anticuerpo anti-STEAP-1 o un inmunoconjugado del mismo en condiciones que faciliten la unión del inmunoconjugado a STEAP-1. "Inhibir el crecimiento o proliferación celular" supone reducir el crecimiento o proliferación de una célula en al menos 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% o 100% e incluye inducir la muerte celular. En ciertas formas de realización, la

célula es una célula tumoral. En ciertas formas de realización, la célula es una célula de próstata, pulmón, colon, vejiga u ovario, o célula de sarcoma de Ewing. En ciertas formas de realización, la célula es un xenotrasplante, por ejemplo, como se ejemplifica en la presente memoria descriptiva.

[0529]. En un aspecto, un anticuerpo o inmunoconjugado de la invención se usa para tratar o prevenir un trastorno proliferativo de células de próstata, pulmón, colon, vejiga u ovario, o célula de sarcoma de Ewing. En ciertas formas de realización, el trastorno de proliferación celular se asocia a un aumento de la expresión o actividad, o ambas, de STEAP-1. Por ejemplo, en ciertas formas de realización, el trastorno proliferativo de células de próstata, pulmón, colon, vejiga u ovario, o células de sarcoma de Ewing se asocia a un aumento de la expresión de STEAP-1 en la superficie de una célula de próstata, pulmón, colon, vejiga u ovario, o célula de sarcoma de Ewing. En ciertas formas de realización, el trastorno proliferativo de células de próstata, pulmón, colon, vejiga u ovario, o célula de sarcoma de Ewing es un tumor o cáncer o metástasis de dicho cáncer.

[0530]. En un aspecto, la invención proporciona métodos para el tratamiento de un trastorno proliferativo de células de próstata, pulmón o colon que comprende la administración a un sujeto de una cantidad eficaz de un anticuerpo anti-STEAP-1 o un inmunoconjugado de éste. En ciertas formas de realización, un método para el tratamiento de un trastorno proliferativo de células de próstata, pulmón o colon supone la administración a un sujeto de una cantidad eficaz de una formulación farmacéutica que comprenda un anticuerpo anti-STEAP-1 o inmunoconjugado anti-STEAP-1 y, opcionalmente, al menos un agente terapéutico adicional, como los que se proporcionan en la presente memoria descriptiva.

[0531]. En un aspecto, al menos algunos de los anticuerpos o inmunoconjugados de la invención se pueden unir a STEAP-1 de especies distintas a la humana. Por consiguiente, los anticuerpos o inmunoconjugados de la invención pueden usarse para unirse a STEAP-1, por ejemplo, en un cultivo celular que contenga STEAP-1, en humanos o en otros mamíferos que tengan un STEAP-1 con el que un anticuerpo o inmunoconjugado de la invención reaccione (por ejemplo, chimpancé, babuino, tití, macaco de Java y macaco de la India, perro, cerdo, rata o ratón). En una forma de realización, un anticuerpo anti-STEAP-1 o inmunoconjugado puede utilizarse para que STEAP-1 actúe sobre las células de próstata, pulmón o colon al poner en contacto un anticuerpo o inmunoconjugado con STEAP-1 para formar un anticuerpo o complejo antígeno-inmunoconjugado, de manera que una citotoxina conjugada del inmunoconjugado ingrese al interior de la célula. En una forma de realización, STEAP-1 a la cual se une el anticuerpo anti-STEAP-1 es un STEAP-1 humano. En una forma de realización, STEAP-1 al cual se une el anticuerpo anti-STEAP-1 es un STEAP-1 de mono cynomolgus. En una forma de realización, el anticuerpo anti-STEAP-1 humanizado se unen al STEAP-1 humano y de mono cynomolgus.

[0532]. En una forma de realización, un anticuerpo anti-STEAP-1 o un inmunoconjugado se puede utilizar en un método para unir STEAP-1 en un sujeto que padezca un trastorno asociado a un

aumento de la expresión y/o actividad de STEAP-1 y dicho método comprende la administración al sujeto del anticuerpo o inmunoconjugado de forma que, en el sujeto, se una el STEAP-1. En una forma de realización, el anticuerpo o inmunoconjugado unido se internaliza en las células de próstata, pulmón, colon, vejiga u ovario o sarcoma de Ewing que expresan STEAP-1. En una forma de realización, STEAP-1 es STEAP-1 humano y el sujeto es un humano. Alternativamente, el sujeto puede ser un mamífero que exprese STEAP-1 al que se une un anticuerpo anti-STEAP-1. Además, el sujeto puede ser un mamífero al que se le ha introducido STEAP-1 (por ejemplo, mediante la administración de STEAP-1 o mediante la expresión de un trasgén que codifique STEAP-1).

[0533]. Un anticuerpo anti-STEAP-1 o un inmunoconjugado puede administrarse a un humano con fines terapéuticos. Además, un anticuerpo anti-STEAP-1 o un inmunoconjugado puede administrarse a mamíferos no humanos que expresan STEAP-1 con el que el anticuerpo reaccione (por ejemplo, primate, perro, cerdo, rata o ratón) para fines veterinarios o como modelo animal de una enfermedad humana. Sobre esto último, dichos modelos animales pueden ser útiles para evaluar la eficacia terapéutica de los anticuerpos o inmunoconjugados de la invención (por ejemplo pruebas de dosis y periodos de administración).

[0534]. Los anticuerpos o inmunoconjugados de la invención se pueden usar solos o junto con otras composiciones en un tratamiento. Por ejemplo, un anticuerpo o inmunoconjugado de la invención se puede administrar conjuntamente con al menos un agente terapéutico adicional y/o adyuvante. En ciertas formas de realización, un agente terapéutico adicional es un agente citotóxico, un agente quimioterapéutico o un agente inhibidor del crecimiento. En una de estas formas de realización, un agente quimioterapéutico es un agente o una combinación de agentes, como por ejemplo, ciclofosfamida, hidroxidaunorubicina, adriamicina, doxorrubicina, vincristina (Oncovin™), prednisolona, CHOP, CVP o COP, o inmunoterapéuticos tales como anti-PSCA (véase, por ejemplo, US6824780)o anti-VEGF (por ejemplo, Avastin®, Genentech, Inc.), anti-HER2 (p-ej., Herceptin®, Omnitarg™ Genentech, Inc.) o anti-HER2 en combinación con Taxol® (véase, por ejemplo, BioWorld Today, 17 de Noviembre de 1999, página 1), donde la terapia de combinación es útil para el tratamiento de trastornos de proliferación celular, cánceres y/o metástasis de cánceres de próstata, pulmón y/o colon..

[0535]. Las terapias combinadas descritas anteriormente incluyen administración combinada (en la que los dos o más agentes se incluyen en la misma o en formulaciones separadas), y administración separada, en cuyo caso, la administración del anticuerpo o inmunoconjugado de la invención puede ocurrir con anterioridad, simultáneamente y/o después de la administración de la terapia o terapias adicionales y/o complementarias. Los anticuerpos o inmunoconjugados de la invención se pueden también usar en combinación con radioterapia.

[0536]. Un anticuerpo o inmunoconjugado de la invención (y agente terapéutico adicional o complementario) puede administrarse por cualquier medio apto, incluyendo las vías parenteral, subcutánea, intraperitoneal, intrapulmonar e intranasal, y, si se desea para tratamiento local, la vía intralesional. Las infusiones parentales incluyen la administración intramuscular, intravenosa, intraarterial, intraperitoneal y subcutánea. Además, el anticuerpo o inmunoconjugadose administra correctamente mediante infusión de efecto rápido con dosis del anticuerpo o inmunoconjugado cada vez menores. La dosificación puede realizarse mediante cualquier vía adecuada, por ejemplo, mediante inyecciones vía intravenosa o subcutánea, dependiendo en parte de si la administración es breve o crónica.

[0537]. La composición de anticuerpos o inmunoconjugados de la invención debería formularse, dosificarse y administrarse de una manera consistente empleando una práctica médica adecuada. Los factores a considerar en este contexto incluyen el trastorno específico sometido a tratamiento, el mamífero específico sometido a tratamiento, el estado clínico del paciente, la causa del trastorno, la localización de la administración del agente, el método de administración, la regulación de la administración y otros factores conocidos por los facultativos. El anticuerpo o inmunoconjugado puede formularse opcionalmente con uno o más agentes utilizados actualmente para prevenir o tratar el trastorno en cuestión. La cantidad eficaz de dichos agentes depende de la cantidad de anticuerpo o inmunoconjugado presente en la formulación, el tipo de trastorno o tratamiento, y otros factores ya abordados con anterioridad. Estos son generalmente usados en las mismas dosis y con las mismas vías de administración descritas en la presente memoria descriptiva o aproximadamente entre 1 y 99% de las dosis descritas en la misma, o en cualquier dosis y mediante cualquier vía empírica y clínicamente considerada apropiada.

[0538]. Para la prevención o tratamiento de la enfermedad, la dosis adecuada de un anticuerpo o inmunoconjugado de la invención (cuando se utiliza aislado o en combinación con uno o más agentes terapéuticos adicionales como los quimioterapéuticos) dependerá del tipo de enfermedad que se tratará, el tipo de anticuerpo o inmunoconjugado, la gravedad y el curso de la enfermedad, de si el anticuerpo o inmunoconjugado se administra para fines preventivos o terapéuticos, la terapia anterior, la historia clínica del paciente y la respuesta al anticuerpo o inmunoconjugado y la discreción del médico tratante. El anticuerpo o inmunoconjugado se administra adecuadamente al paciente una vez o en una serie de tratamientos. Dependiendo del tipo y la gravedad de la enfermedad, una dosis inicial candidata para la administración en un paciente es de aproximadamente 1 μg/kg a 100 mg/kg (por ej. 0,1mg/kg-20mg/kg) de anticuerpo o inmunoconjugado, ya sea, por ejemplo, mediante una o más administraciones separadas o mediante una infusión continua. Una dosis diaria normal puede oscilar entre aproximadamente 1 μg/kg a 100 mg/kg o más, dependiendo de los factores anteriormente mencionados. Para administraciones repetidas durante varios días o más, dependiendo de la enfermedad, el tratamiento se mantendría, por lo general, hasta que ocurriese una supresión deseada de los síntomas de la misma. Un ejemplo de dosis del

anticuerpo o inmunoconjugado se encontraría en el intervalo de aproximadamente 0,05 mg/kg y aproximadamente 10 mg/kg. Por lo tanto, se puede administrar al paciente una o más dosis de aproximadamente 0,5 mg/kg, 2,0 mg/kg, 4,0 mg/kg o 10 mg/kg (o cualquier combinación de las mismas) de anticuerpo o inmunoconjugado. Dichas dosis pueden administrarse de manera intermitente, por ejemplo, una vez por semana o cada tres semanas (por ejemplo, de modo que el paciente reciba aproximadamente de dos a veinte, o por ejemplo, aproximadamente seis dosis del anticuerpo o inmunoconjugado). Se puede administrar una mayor carga de dosis inicial seguida de una o más dosis menores. Un ejemplo de pauta posológica comprende la administración de una dosis inicial aproximada de 4 mg/kg, seguida de una dosis de mantenimiento semanal de aproximadamente 2 mg/kg del anticuerpo. No obstante, también pueden ser útiles otras pautas posológicas. El progreso de este tratamiento se controla fácilmente mediante técnicas y ensayos convencionales.

Ensayos

[0539]. Los anticuerpos anti-STEAP-1 o inmunoconjugados de la invención pueden caracterizarse por sus propiedades físico-químicas o por sus actividades biológicas, o ambas, mediante varios ensayos conocidos en la especialidad.

Ensayos de actividad

[0540]. En un aspecto, se proporcionan ensayos para la identificación de anticuerpos anti-STEAP-1 o inmunoconjugados de los mismos con actividad biológica. La actividad biológica puede incluir, por ejemplo, la capacidad de inhibir el crecimiento o proliferación celular (por ejemplo, actividad de "destrucción celular"), o la capacidad de inducir la muerte celular, incluida la muerte celular programada (apoptosis). También se proporcionan anticuerpos o inmunoconjugados con dicha actividad biológica *in vivo* y/o *in vitro*.

[0541]. En ciertas formas de realización, se comprueba la capacidad de un anticuerpo anti-STEAP-1 o un inmunoconjugado de éste para inhibir el crecimiento o proliferación celular *in vitro*. Los ensayos para la inhibición del crecimiento o proliferación celular son bien conocidos en la materia. Determinados ensayos para la proliferación celular, ejemplificados mediantes ensayos "de destrucción de celulas" descritos en la presente memoria descriptiva, miden la viabilidad celular. Uno de estos ensayos es el ensayo de viabilidad celular por luminiscencia CellTiter-Glo™, que está comercialmente disponible en Promega (Madison, WI). Este ensayo determina el número de células viables en cultivo basado en cuantificación de ATP presente, que indica las células activas metabólicamente. Véase Crouch y col., (1993) *J. Immunol. Meth.* 160:81-88, patente de EE.UU: N.º 6602677. El ensayo puede realizarse en un formato de 96 ó 384 pocillos, lo que hace posible su uso para detección automatizada de alto rendimiento (HTS). Véase Cree y col., (1995) *AntiCancer Drugs* 6:398-404. El procedimiento de este ensayo supone añadir un único reactivo (Reactivo CellTiter-Glo®) directamente a las células en el cultivo. Ello da lugar a

lisis celular y generación de una señal luminescente producida por una reacción con luciferasa. La señal luminiscente es proporcional a la cantidad de ATP presente, que es directamente proporcional al número de células viables presentes en el cultivo. Los datos se pueden registrar mediante un luminómetro o un dispositivo de captación de imágenes mediante cámara CCD. La producción de luminiscencia se expresa como unidades de luz relativas (RLU).

[0542]. Otro ensayo para la proliferación celular es el ensayo "MMT" un ensayo colorimétrico que mide la oxidación de bromuro 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium a formazan mediante reductasa mitocondrial. Como el ensayo CellTiter-Glo™, este ensayo indica el número de células metabólicamente activas presentes en un citocultivo. Véase, por ejemplo, Mosmann (1983) *J. Immunol. Meth.* 65:55-63, y Zhang y col. (2005) *Cancer Res.* 65:3877-3882.

[0543]. En un aspecto, se comprueba la capacidad de un anticuerpo anti-STEAP-1 para inducir muerte celular *in vitro*. Los ensayos para la inducción de la muerte celular son bien conocidos en la materia. En algunas formas de realización, estos ensayos miden, por ejemplo, la pérdida de integridad de la membrana, como se indica mediante absorción de yoduro de propidio (PI), azul de tripan (véase Moore y col. (1995) *Cytotechnology*, 17:1-11), o 7AAD. En un ensayo ejemplar de absorción de PI, las células se cultivan en medio Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) enriquecido con 10% de FBS inactivado por calor (Hyclone) y 2 mM de L-glutamina. De este modo, el ensayo se realiza en ausencia de complemento e inmunocitos efectores. Las células se siembran a una densidad de 3 x 10^6 por placa en placas de 100 x 20 mm y se deja que se emparejen durante la noche. Después el medio se retira y se reemplaza con un medio nuevo solo o que contenga diversas concentraciones del anticuerpo o inmunoconjugado. Las células se incuban durante 3 días. Tras el tratamiento, las monocapas se lavan con PBS y se separan mediante tripsinización. A continuación, las células se centrifugan a 1200 rpm durante 5 minutos a 4 °C; el pellet se resuspende en 3 ml de solución tamponada fría de unión a Ca^{2+} (10 mM Hepes, pH 7,4, 140 mM NaCl, 2,5 mM $CaCl_2$) y se alicuotan en tubos de 35 mm con filtro de 12 x 75 mm (1 ml por tubo, 3 tubos por grupo de tratamiento) para la eliminación de la agregación celular. Los tubos reciben entonces el PI (10 µg/ml). Las muestras se analizan utilizando un citómetro de flujo FACSCAN™ y software FACSCONVERT™ CellQuest (Becton Dickinson). Se identifican así los anticuerpos o inmunoconjugados que inducen niveles estadísticamente significativos de muerte celular, como se determina mediante absorción de PI.

[0544]. En un aspecto, se comprueba la capacidad de un anticuerpo anti-STEAP-1 o inmunoconjugado para inducir la apoptosis (muerte celular programada) *in vitro*. Un ensayo ejemplar para anticuerpos o inmunoconjugados que inducen la apoptosis es un ensayo de unión a anexina. En un ensayo ejemplar de unión a anexina, las células se cultivan y se siembran en placas tal y como se describe en el párrafo anterior. Después el medio se retira y se reemplaza con un medio nuevo solo o que contenga de

0,001 a 10 μg/ml del anticuerpo o inmunoconjugado. Después de un período de incubación de tres días, las monocapas se lavan con PBS y se separan por tripsinización. Después, las células se centrifugan, se resuspenden en un tampón de unión a Ca^{2+} y se alicuotan en tubos tal y como se describe anteriormente. Después, se añade a los tubos la anexina marcada (por ejemplo, anexina V-FTIC) (1 μg/ml). Las muestras se analizan utilizando un citómetro de flujo FACSCAN™ y software FACSCONVERT™ CellQuest (BD Biosciences). Se identifican así los anticuerpos o inmunoconjugados que inducen niveles estadísticamente significativos de unión a anexina con respecto a controles. Otro ensayo ejemplar para anticuerpos o inmunoconjugados que inducen apoptosis es un ensayo colorimétrico ELISA con complejos ADN-histonas para la detección de degradación internucleosomal de ADN genómico. Este ensayo se puede llevar a cabo utilizando, por ejemplo, el Kit ELISA de detección de muerte celular (Roche, Palo Alto, CA).

[0545]. Las células para uso en cualquiera de los ensayos *in vitro* anteriores incluyen células o líneas celulares que expresan naturalmente STEAP-1 o que se han diseñado para expresar STEAP-1. Estas células incluyen células tumorales que sobreexpresan STEAP-1 con respecto a células normales del mismo origen tisular. Estas células también incluyen líneas celulares (incluidas líneas celulares tumorales) que expresan STEAP-1 y líneas celulares que normalmente no expresan STEAP-1 pero han sido transfectadas con ácido nucleico que codifica STEAP-1.

[0546]. En un aspecto, se comprueba la capacidad de un anticuerpo anti-STEAP-1 o un inmunoconjugado del mismo para inhibir el crecimiento o proliferación celular *in vivo*. En ciertas formas de realización, se comprueba la capacidad de un anticuerpo anti-STEAP-1 o un inmunoconjugado del mismo para inhibir el crecimiento tumoral *in vivo*. Los sistemas de modelos *in vivo*, como modelos xenotrasplantados, se pueden utilizar para estas pruebas. En un sistema xenotrasplantado ejemplar, las células tumorales humanas se introducen en un animal no humano inmunodeprimido apto, por ejemplo, un ratón SCID. Se administra al animal un anticuerpo o inmunoconjugado de la invención. Se mide la capacidad del anticuerpo o inmunoconjugado para inhibir o reducir el crecimiento tumoral. En ciertas formas de realización del sistema xenotrasplantado anterior, las células tumorales humanas son células tumorales procedentes de un paciente humano. Dichas células útiles para la preparación de modelos xenostrasplantados incluyen líneas celulares de tumor de próstata, pulmón o colon humano, que comprenden, aunque sin limitarse a ellas, las células PC3 que expresan STEAP-1 exógeno, y células que expresan naturalmente STEAP-1 que incluyen, sin limitación, las células LnCAP (Southern Research Institute, Birmingham, AL), las células LuCAP 77 y las células LuCAP35V (University of Washington, Seattle, WA). En ciertas formas de realización, las células tumorales humanas se introducen en un animal no humano debidamente inmunodeprimido mediante inyección subcutánea o mediante trasplante en un sitio apto, como panículos adiposos mamarios.

Ensayos de unión y otros ensayos

[0547]. En un aspecto, se prueba la actividad de unión de un anticuerpo anti-STEAP-1 al antígeno. Por ejemplo, en ciertas formas de realización, se comprueba la capacidad de un anticuerpo anti-STEAP-1 para unirse a STEAP-1 exógeno o endógeno expresado en la superficie de una célula. Para realizar esta prueba puede utilizarse un ensayo FACS.

[0548]. En un aspecto, los ensayos de competición pueden utilizarse para identificar un anticuerpo monoclonal que compite con un injerto 120 o variantes de ellos humanizados, incluyendo, entre otros, un anticuerpo 120v.24 para que se unan a STEAP-1. En ciertas formas de realización, este anticuerpo competitivo se une al mismo epitopo (por ejemplo, un péptido del epítopo lineal o un epitopo conformacional formado por la expresión de STEAP-1 en una superficie celular) que se une mediante un anticuerpo del injerto 120, o un anticuerpo humanizado del injerto 120, incluyendo la variante anticuerpo humanizado 120v.24 anti-STEAP-1. Entre los ensayos de competición ejemplares se incluyen, entre otros, los ensayos habituales como los indicados en Harlow y Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Métodos ejemplares detallados para el mapeo de un epítopo al que se une un anticuerpo se señalan en Morris (1996) "Epitope Mapping Protocols," en *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). Se dice que dos anticuerpos se unen al mismo epitopo si cada uno bloquea la unión del otro en un 50% o más.

[0549]. En un ensayo de competición ejemplar, un STEAP-1 inmovilizado se incuba en una solución que comprenda un primer anticuerpo marcado que se una a STEAP-1 (por ejemplo, un anticuerpo murino 120.545, un anticuerpo del injerto 120, o un anticuerpo humanizado 120v.24) y un segundo anticuerpo sin marcar del que se comprueba su capacidad para competir con el primero para su unión a STEAP-1. El segundo anticuerpo puede estar presente en un sobrenadante de hibridoma. Como control, se incuba un STEAP-1 inmovilizado en una solución que contenga el primer anticuerpo marcado pero no el segundo anticuerpo no marcado. Tras la incubación en condiciones que favorezcan la unión del primer anticuerpo a STEAP-1, se elimina el anticuerpo no unido sobrante y se mide la cantidad de marcador asociada a STEAP-1 inmovilizado. Si la cantidad de marcador asociada a STEAP-1 inmovilizado es sustancialmente reducida en la muestra de prueba con respecto a la muestra de control, esto indica que el segundo anticuerpo está compitiendo con el primero para la unión a STEAP-1. En ciertas formas de realización, un STEAP-1 inmovilizado está presente en la superfice de una célula o en una preparación de membrana obtenida a partir de una célula que exprese STEAP-1 en su superficie.

[0550]. En un aspecto, los anticuerpos purificados anti-STEAP-1 se pueden caracterizar además mediante una serie de ensayos que incluyen, entre otros, la secuenciación del extremo N-terminal, el análisis de aminoácidos, la cromatografía líquida de alta resolución no desnaturalizante de exclusión por tamaño (HPLC), la espectrometría de masas, la cromatografía de intercambio iónico y la digestión con papaína.

[0551]. En una forma de realización, la invención contempla un anticuerpo alterado que tiene algunas, pero no la totalidad, de las funciones efectoras, lo que le convierte en un candidato deseable para muchas aplicaciones en las que la semivida del anticuerpo *in vivo* es importante, pero determinadas funciones efectoras (como complemento y ADCC) son innecesarias o dañinas. En ciertas formas de realización, se miden las actividades Fc del anticuerpo para asegurar que sólo se mantienen las propiedades deseadas. Se pueden llevar a cabo ensayos de citotoxicidad *in vitro* o *in vivo* para confirmar la reducción/disminución de actividades de CDC o ADCC, o ambos. Por ejemplo, los ensayos de unión del receptor Fc (FcR) pueden realizarse para asegurar que el anticuerpo carece de unión a FcγR (por eso es probable que carezca de actividad ADCC), pero conserva la capacidad de unión a FcRn. Las células primarias para mediar en la ADCC, células NK, expresan sólo Fc(RIII, mientras que los monocitos expresan Fc(RI, Fc(RII y Fc(RIII. La expresión del FcR en células hematopoyéticas se resume en la Tabla 3 de la página 464 de Ravetch y Kinet, *Annu. Rev. Immunol*, 9:457-92 (1991). Se describe un ejemplo de un ensayo *in vitro* para evaluar la actividad ADCC de una molécula de interés en la patente de EE.UU. N.° 5.500.362 ó 5.821.337. Entre las células efectoras útiles para tales ensayos se encuentran las células mononucleares de la circulación periférica (PBMC) y las células asesinas (NK). Como alternativa, o adicionalmente, la actividad de ADCC de la molécula de interés se puede evaluar *in vivo*, por ejemplo, en un modelo animal como el que se desvela en Clynes y col., *PNAS (USA)*, 95:652-656 (1998). También se pueden llevar a cabo los ensayos de unión C1q para confirmar que el anticuerpo no puede unirse al C1q y por ello carece de actividad CDC. A fin de evaluar la activación del complemento, se puede efectuar un ensayo de CDC, por ejemplo, tal como se describe en Gazzano-Santoro y col., *J. Immunol. Methods*, 202:163 (1996). También se pueden realizar la unión de FcRn y eliminación *in vivo*/determinaciones de semivida con el uso de métodos conocidos en la materia.

EJEMPLOS

[0552]. A continuación se muestran ejemplos de los métodos y composiciones de la invención. A partir de la descripción general proporcionada anteriormente, se entiende que se pueden dar varias formas de realización.

Ejemplo 1: Preparación de anticuerpos anti-STEAP-1 humanizados

[0553]. Las moléculas de ácidos nucleicos que codifican variantes de las secuencias de aminoácidos del anticuerpo, fragmento de anticuerpo o dominios de la VL o VH se preparan mediante una variedad de procedimientos conocidos en la materia. Estos procedimientos incluyen el aislamiento de una fuente natural (en el caso de variantes de las secuencias de aminoácidos producidas de forma natural) o la preparación por mutagénesis mediada por oligonucleótidos (o dirigida al sitio), la mutagénesis dirigida mediante PCR y la mutagénesis por inserción de un casete de una variante preparada anteriormente o una versión no variante del anticuerpo, fragmento de anticuerpo o dominios de la VL o VH. Por ejemplo, se

pueden crear bibliotecas actuando sobre posiciones aminocídicas accesibles del VL en el VH, y opcionalmente en una o más CDR, para sustitución de aminoácidos por variantes de aminoácidos utilizando el método Kunkel. Véase, por ejemplo, Kunkel y col., *Methods Enzymol.* (1987), 154:367-382 y los ejemplos de la presente memoria descriptiva. La generación de secuencias aleatorizadas también se describe más adelante en los ejemplos.

[0554]. La secuencia de oligonucleótidos incluye uno o más de los conjuntos de codones diseñados para una posición concreta en una región CDR (HVR) o FR de un polipéptido de la invención. Un conjunto de codones es un grupo de distintas secuencias de tripletes de nucleótidos usados para codificar variantes de aminoácidos deseadas. Los conjuntos de codones se pueden representar mediante símbolos para designar a nucleótidos concretos o a mezclas equimolares de nucleótidos, como se muestra a continuación según el código IUB.

[0555]. **CÓDIGOS IUB**

G Guanina

A Adenina

T Timina

C Citosina

R (A o G)

Y (C o T)

M (A o C)

K (G o T)

S (C o G)

W (A o T)

H (A o C o T)

B (C o G o T)

V (A o C o G)

D (A o G o T)

N (A o C o G o T)

[0556]. Por ejemplo, en el conjunto de codones DVK, D puede ser los nucleótidos A o G o T; V puede ser A o G o C; y K puede ser G o T. Este conjunto de codones puede presentar 18 codones diferentes y puede codificar los aminoácidos Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly y Cys.

[0557]. Se pueden sintetizar conjuntos de cebadores u oligonucleótidos utilizando métodos estándar. Es posible sintetizar un conjunto de oligonucleótidos mediante síntesis durante la fase sólida, por ejemplo, que contiene secuencias que representan todas las posibles combinaciones de tripletes de

nucleótidos proporcionadas por el conjunto de codones y que codificará el grupo deseado de aminoácidos. La síntesis de oligonucleótidos con "degeneración" de los nucleótidos seleccionados en determinadas posiciones es bien conocida en la materia. Estos conjuntos de nucleótidos con determinados conjuntos de codones pueden sintetizarse mediante sintentizadores de ácidos nucleicos comercializados (disponibles, por ejemplo, en Applied Biosystems, Foster City, CA) o se pueden adquirir (por ejemplo, a Life Technologies, Rockville, MD). Por lo tanto, un conjunto de oligonucleótidos sintetizados que tenga un conjunto concreto de codones incluirá habitualmente una pluralidad de oligonucleótidos con diferentes secuencias, y estas diferencias estarán establecidas por el conjunto de codones dentro de la secuencia total. Los oligonucleótidos, según se utilizan en la presente invención, tienen secuencias que permiten la hibridación de una plantilla de ácidos nucleicos de dominio variable y también pueden incluir sitios con enzimas de restricción para fines de clonación.

[0558]. En un método, se pueden crear secuencias de ácidos nucleicos que codifican variantes de aminoácidos mediante mutagénesis mediada por oligonucleótidos. Esta técnica es bien conocida en la materia y fue descrita por Zoller y col, 1987, *Nucleic Acids Res.* 10:6487-6504. Explicado brevemente, se crean secuencias de ácidos nucleicos que codifican variantes de ácidos nucleicos mediante hibridación de un conjunto de oligonucleótidos que codifican los conjuntos de codones necesarios con una plantilla de ADN, donde la plantilla es la forma de una única cadena del plásmido que contiene una secuencia de la plantilla de ácidos nucleicos de la región variable. Tras la hibridación, se utiliza ADN polimerasa para sintetizar una segunda cadena complementaria completa de la plantilla, que, de este modo, incorporará el cebador oligonucleótido y contendrá los conjuntos de codones proporcionados por el conjunto oligonucleótido.

[0559]. Por lo general, se utilizan oligonucleótidos de al menos 25 nucleótidos. Un oligonucleótido óptimo tendrá entre 12 y 15 nucleótidos que son completamente complementarios con respecto a la plantilla en cada lado de la codificación del nucleótido o nucleótidos para la mutación o mutaciones. Esto garantiza que la hibridación del oligonucleótido con la molécula de la plantilla de ADN de cadena única se llevará a cabo correctamente. Los oligonucleótidos se sintetizan fácilmente utilizando técnicas bien conocidas en la materia, como la descrita por Crea y col., *Proc. Nat'l. Acad. Sci. USA*, 75:5765 (1978).

[0560]. La plantilla de ADN está bien generada por aquellos vectores que se derivan de los vectores bacteriófagos M13 (son adecuados los vectores M13mp18 y M13mp19 comercializados) bien por aquellos vectores que contienen un origen de replicación de fagos de cadena única, tal como se describe en Viera y col., *Meth. Enzymol.*, 153:3 (1987). De este modo, el ADN que va a mutarse se puede insertar en uno de estos vectores con el fin de generar una plantilla de cadena única. La producción de la plantilla de cadena única se describe en los apartados 4.21-4.41 de Sambrook y col., mencionado anteriormente.

[0561]. Para alterar la secuencia de ADN nativo, el oligonucleótido se hibrida con la plantilla de cadena única en condiciones de hibridación apropiadas. A continuación, se añade una enzima polimerizadora del ADN, por lo general la ADN polimerasa T7 o el fragmento Klenow de la ADN polimerasa I, para sintetizar la cadena complementaria de la plantilla utilizando el oligonucleótido como cebador para la síntesis. Por lo tanto, una molécula heterodúplex se forma de tal manera que una cadena de ADN codifica la forma mutada del gen 1 y otra cadena (la plantilla original) codifica la secuencia inalterada nativa del gen 1. Esta molécula heterodúplex se transforma en una célula hospedera apta, por lo general, una procariota tal como *E. coli* JM101. Tras cultivar las células, se colocan en placas de agarosa y se analizan utilizando el cebador oligonucleótido radiomarcado con 32-fosfato para identificar las colonias bacterianas que contienen el ADN mutado.

[0562]. El método que se acaba de describir se puede modificar, de forma que se cree una molécula homodúplex en la que las dos cadenas del plásmido contengan la mutación o mutaciones. Las modificaciones son las siguientes: El oligonucleótido de cadena única es hibridado con la plantilla de cadena única como se ha descrito anteriormente. Se combina una mezcla de tres desoxirribonucleótidos, desoxirriboadenosina (dATP), desoxirriboguanosina (dGTP) y desoxirribotimidina (dTT), con una tiodesoxirribocitosina modificada denominada dCTP-(aS) (que se puede obtener de Amersham). Esta mezcla se añade al complejo formado por el oligonucleótido y la plantilla. Al añadir a la mezcla la ADN polimerasa, se genera una cadena de ADN idéntica a la plantilla, salvo por las bases mutadas. Además, esta nueva cadena de ADN contendrá dCTP-(aS) en vez de dCTP, que sirve para protegerla de la digestión por endonucleasa de restricción. Una vez que se hace una muesca en la cadena de la plantilla del heterodúplex de doble cadena utilizando una enzima de restricción apropiada, la cadena de la plantilla se puede digerir con nucleasa ExoIII u otra nucleasa apropiada pasada la región que contiene el sitio o los sitios que se van a mutagenizar. A continuación, se detiene la reacción para dejar una molécula que sea sólo parcialmente de cadena única. Entonces, se forma un homodúplex de ADN de doble cadena completo utilizando ADN polimerasa en presencia de los cuatro trifosfatos desoxirribonucleótidos, ATP y ADN ligasa. Esta molécula homodúplex se puede transformar entonces en una célula hospedera apropiada.

[0563]. Como se ha indicado anteriormente, la secuencia del conjunto de oligonucleótidos tiene longitud suficiente para hibridar con el ácido nucleico de la plantilla y también puede contener sitios de restricción, aunque no es necesario que lo haga. La plantilla de ADN se puede generar mediante aquellos vectores que se derivan de los vectores bacteriófagos M13 o mediante aquellos vectores que contienen un origen de replicación del fago de cadena única, como se describe en Viera y col. ((1987) *Meth. Enzymol.*, 153:3). De este modo, el ADN que va a mutarse se debe insertar en uno de estos vectores con el fin de generar una plantilla de cadena única. La producción de la plantilla de cadena única se describe en los apartados 4.21-4.41 de Sambrook y col., mencionado anteriormente.

[0564]. Según otro método, se puede generar una biblioteca proporcionando conjuntos de oligonucleótidos situados antes del extremo 5' del promotor y después del extremo 3' del gen, de forma que cada conjunto tenga una pluralidad de oligonucleótidos con secuencias diferentes y las distintas secuencias establecidas por los conjuntos de codones estén dentro de la secuencia de los oligonucleótidos. Los conjuntos de oligonucleótidos situados antes del extremo 5' del promotor y después del extremo 3' del gen, junto con una secuencia de ácidos nucleicos de la plantilla del dominio variable, se pueden utilizar en una reacción en cadena de la polimerasa para generar una "biblioteca" de productos obtenidos mediante PCR. Se puede hacer referencia a los productos obtenidos mediante PCR como "casetes de ácidos nucleicos", puesto que se pueden fusionar con otras secuencias de ácidos nucleicos afines o no afines, por ejemplo, proteínas de la cápside de un virus y dominios de dimerización, utilizando técnicas reconocidas en biología molecular.

[0565]. Se pueden utilizar conjuntos de oligonucleótidos en una reacción en cadena de la polimerasa usando una secuencia de la plantilla de ácidos nucleicos de un dominio variable como la plantilla para crear casetes de ácidos nucleicos. La secuencia de la plantilla de ácidos nucleicos del dominio variable puede ser cualquier porción de cadenas pesadas de inmunoglobulinas que contengan las secuencias de ácidos nucleicos diana (es decir, secuencias de ácidos nucleicos que codifican aminoácidos destinados a sustitución). La secuencia de la plantilla de ácidos nucleicos de la región variable es una porción de una molécula de ADN de doble cadena con una primera hebra de ácido nucleico y una segunda hebra complementaria de ácido nucleico. La secuencia de la plantilla de ácidos nucleicos del dominio variable contiene al menos una porción de un dominio variable y tiene al menos una CDR. En algunos casos, la secuencia de la plantilla de ácidos nucleicos del dominio variable contiene más de una CDR. Una porción situada antes del extremo 5' y una porción situada después del extremo 3' de la secuencia de la plantilla de ácidos nucleicos del dominio variable puede destinarse a hibridación con miembros de un conjunto de oligonucleótidos situados antes del extremo 5' y de un conjunto de oligonucleótidos situados después del extremo 3'.

[0566]. Se puede hibridar un primer oligonucleótido del conjunto de cebadores situados antes del extremo 5' con la primera cadena de ácido nucleico y un segundo oligonucleótido del conjunto de cebadores situados después del extremo 3' con la segunda cadena de ácido nucleico. Los cebadores de oligonucleótidos pueden incluir uno o más conjuntos de condones y estar diseñados para hibridar con una porción de la secuencia de la plantilla de ácidos nucleicos de la región variable. El uso de estos oligonucleótidos puede introducir dos o más conjuntos de codones en el producto obtenido mediante PCR (es decir, el casete de ácido nucleico) tras la PCR. El cebador oligonucleótido que hibrida con regiones de la secuencia de ácidos nucleicos que codifican el dominio variable de un anticuerpo incluye porciones que codifican residuos de la CDR que están destinados a sustitución de aminoácidos.

[0567]. También se pueden sintetizar los conjuntos de oligonucleótidos situados antes del extremo 5' y después del extremo 3' para incluir sitios de restricción dentro de la secuencia de oligonucleótidos. Estos sitios de restricción pueden facilitar la inserción de las casetes de ácido nucleico (es decir, los productos de la reacción PCR) en un vector de expresión que tenga una secuencia adicional de anticuerpos. En una forma de realización, los sitios de restricción están diseñados para facilitar la clonación de las casetes de ácido nucleico sin introducir secuencias de ácidos nucleicos externas ni eliminar las secuencias originales de ácidos nucleicos de regiones estructurales o CDR.

[0568]. Las casetes de ácido nucleico pueden clonarse para obtener cualquier vector apto para la expresión de una porción o la totalidad de la secuencia de la cadena pesada o ligera que contenga las sustituciones de aminoácidos dirigidas generadas mediante la reacción PCR. Según los métodos detallados en la invención, la casete de ácidos nucleicos se clona para formar un vector que facilite la producción de una porción o la totalidad de la secuencia de la cadena pesada o ligera fusionada con parte o la totalidad de una proteína de la cápside de un virus (es decir, creando una proteína de fusión) y representada en la superficie de una partícula o célula. Aunque están disponibles diversos tipos de vectores que se pueden utilizar para realizar prácticas de esta invención, los vectores fagémidos son los preferidos para uso en la presente memoria descriptiva, dado que se pueden construir con relativa sencillez y se pueden amplificar fácilmente. Los vectores fagémidos suelen contener diversos componentes, que incluyen promotores, secuencias de señal, genes de selección fenotípica, sitios de origen de la replicación y otros componentes necesarios, que son conocidos por los expertos en la materia.

[0569]. Cuando se desea expresar una combinación concreta de variantes de aminoácidos, la casete de ácidos nucleicos contiene una secuencia que puede codificar parte o la totalidad del dominio variable de la cadena ligera o pesada y puede codificar las combinaciones de las variantes de aminoácidos. Para la producción de anticuerpos que contengan estas variantes de aminoácidos o combinaciones de variantes de aminoácidos, como en una biblioteca, las casetes de ácidos nucleicos se pueden insertar en un vector de expresión que contenga una secuencia adicional de anticuerpos, por ejemplo, la totalidad o porciones de los dominios constante o variable de las regiones variables de la cadena pesada y ligera. Estas secuencias adicionales de anticuerpos también se pueden fusionar con otras secuencias de ácidos nucleicos, como secuencias que codifican proteínas de la cápside de un virus, y de este modo permitir la producción de una proteína de fusión.

[0570]. En la presente memoria se describe la humanización del anticuerpo STEAP-1 antihumano murino.

Materiales y métodos

[0571]. La numeración de los residuos se realiza de acuerdo con Kabat (Kabat *y col.*, *Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of

Health, Bethesda, MD (1991)). Para las abreviaturas de los aminoácidos se usa una sola letra. Las degeneraciones de ADN se representan usando el código IUB (N = A/C/G/T, D = A/G/T, V = A/C/G, B= C/G/T, H= A/C/T, K = G/T, M = A/C, R = A/G, S = G/C, W= A/T, Y = C/T).

[0572]. ***Clonación de dominios variables 120 murinos y generación de un anticuerpo 120 quimérico*** – Se extrajo el ARN total de las células de hibridoma que producen M2-120.545 (designadas en esta memoria como "murino 120" o "mu120") usando métodos estándar. El dominio variable ligero (VL) y el dominio variable pesado (VH) se amplificaron usando RT-PCR con los cebadores degenerados de las cadenas ligera y pesada. Los cebadores anversos eran específicos para la secuencia de aminoácidos N-terminal de las regiones VL y VH. Los cebadores reversos de las cadenas ligera y pesada, respectivamente, se diseñaron para hibridar en una región del dominio constante ligero (CL) y del dominio constante pesado 1(CH1), y que se conservan en gran medida entre las especies. Las VL y VH amplificadas se clonaron en vectores de expresión de mamífero. La secuencia de polinucleótidos en las inserciones se determinó mediante los métodos de secuenciación habituales. Las secuencias de aminoácidos M2-120.545 ("mu 120") VL y VH se muestran en las Figuras 2A y 2B, respectivamente.

[0573]. *Generación de quimera murina 120* – Se preparó un anticuerpo anti-STEAP-1 quimérico mediante la fusión de regiones variables pesadas (VH) y variables ligeras (VL) murino 120 con los dominios constantes de un IgG humano. El anticuerpo resultante se designa en esta memoria como "120 quimera", "quimera 120", "IgG 120 quimérico" o "quimera Fc".

[0574]. ***Injertos directos de regiones hipervariables en estructura de consenso humana del aceptor*** – Las variantes construidas durante la humanización de murino 120 se evaluaron tanto como proteína en la forma de un IgG como de un Fab representado en un fago.

[0575]. El uso fagémido para este trabajo es un vector para representación de Fab-g3 monovalente y consiste en dos estructuras de lectura abierta bajo control del promotor phoA. La primera estructura de lectura abierta consiste en una secuencia de señal StII fusionada con los dominios VL y CH1 de la cadena ligera del aceptor y la segunda consiste en la secuencia de señal stII fusionada con los dominios VH y CH1 de la cadena pesada del aceptor seguida por la proteína de la cápside del fago menor P3.

[0576]. Los dominios VL y VH del murino 120 se alinearon con las secuencias de consenso de los subgrupos VH III humano (huIII) y kappa I VL humano (huKI). Para realizar los injertos CDR, se injertaron regiones hipervariables del anticuerpo murino 120 en las estructuras del aceptor huKI y huIII.

[0577]. Las regiones hipervariables del anticuerpo murino 120 (mu120) se diseñaron en la estructura de consenso humana del aceptor para generar el injerto CDR directo (designado en esta memoria como "120 injerto" o "injerto 120"). En el dominio VL de las siguientes regiones se injertaron al aceptor de

consenso humano: las posiciones 24-34 (L1), 50-56 (L2) y 89-97 (L3). En el dominio VH, se injertaron las posiciones 26-35a (H1), 49-65 (H2) y 95-102 (H3). Las secuencias de las regiones variables de las cadenas ligera y pesada del injerto 120 se muestran en las Figuras 2A-2B. Los CDR (también designados en esta memoria como HVR) se muestran en recuadros (Figuras 2A-2B). Estas definiciones CDR incluyen posiciones definidas por su hipervariabilidad de secuencia (ref. Kabat), su localización estructural (ref. Chothia) y su implicación en los contactos antígeno-anticuerpo (MacCallum *y col. J. Mol. Biol.* 262: 732-745 (1996)).

[0578]. Las variantes de injerto directo expresadas como un Fab expresado en un fago o como un IgG se generaron mediante mutagénesis según el método de Kunkel usando un oligonucleótido separado para cada región hipervariable. Los clones correctos se evaluaron mediante la secuenciación del ADN.

[0579]. ***Generación de variantes de fago 120 humanizadas*** – Las variantes 120 humanizadas se generaron como Fab expresado en un fago mediante mutagénesis según el método de Kunkle. Se añadió un oligonucleótido fosforilado a un molde de Kunkel de 300 ng en Tris 50 mM pH 7,5, $MgCl_2$ 10 mM en un volumen final de 10 µl. La mezcla fue hibridada a 90°C durante 2 min., a 50°C durante 5 min. y después enfriada sobre hielo. Luego se procedió a llenar el molde hibridado añadiendo 0,5 µl de ATP 10mM, 0,5 µl dNTPs 10mM (10mM cada uno de dATP, dCTP, dGTP y dTTP), 1 µl de DTT 100mM, 1 µl de 10X TM tampón (Tris 0,5 M pH 7,5, $MgCl_2$ 0,1 M), 80 U T4 ligasa, y 4 U T7 polimerasa en un volumen total de 20 µl durante 2 horas a temperatura ambiente. A continuación, una vez llenado y ligado el producto se lo transformó en células azules XL1 (Stratagene). Los clones correctos se identificaron mediante la secuenciación del ADN.

[0580]. Los clones de fago correctos se desarrollaron en 25 ml de 2YT conteniendo 50 µg/ml de carbenacilina y fago auxiliar M13/KO7 (MOI 10) durante toda la noche a 37 °C.

[0581]. ***Evaluación de las variantes 120 humanizadas*** – Las variantes humanizadas expresadas como IgG se evaluaron mediante análisis FACS utilizando líneas celulares positivas (293 Steap1 NT LB50) y negativas (293 vector S408) de STEAP-1.

[0582]. Las variantes humanizadas expresadas como Fab expresado en fago también se evaluaron mediante análisis FACS. Las variantes de Fab expresado en fago se evaluaron primero por su nivel de expresión de Fab usando un ensayo ELISA de fagos para detectar un señalizador fusionado a la cadena ligera del Fab. Las placas de microtitulación se cubrieron con anti-gD 1766 a 10 µg/ml en PBS durante toda la noche y después se bloquearon con un bloqueante de caseína. Los fagos procedentes de los sobrenadantes del cultivo fueron diluidos serialmente en PBST conteniendo BSA al 0,5% en una placa de microtitulación de cultivo de tejido y transferidos a los pocillos recubiertos durante 1 hora para capturar el Fab expresado en fago. La placa se lavó con PBST y se añadió anti-M13 (Amersham Pharmacia Biotech) conjugado con HRP (1:5000 en PBST conteniendo BSA al 0,5 %) durante 40 minutos. La placa se lavó con

PBST y se desarrolló añadiendo sustrato de tetrametilbencidina (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Se usó la absorbancia a 405 nm como una estimación del nivel de expresión del Fab en la superficie del fago. Durante la expresión las preparaciones de fago se normalizaron mediante dilución. Para el análisis FACS se usaron fagos puros con baja expresión (p.ej. los quimera).

[0583]. Para el análisis FACS de unión de fagos, se procedió a sacar las células de la placa usando EDTA 2 mM, a recogerlas en un tubo de base cónica de 15 ml y a granularlas mediante centrifugación. Se volvió a suspender las células (5 X 10^5 células por muestra) en 100 µl de fago (normalizado por nivel de expresión) en tampón FACS (FBS al 1%, PBS con EDTA 2 mM) y se las incubó durante 1-2 horas sobre hielo. Las muestras fueron lavadas dos veces con tampón FACS mediante centrifugación. Se añadió un anticuerpo de control anti-M13 5G7 (Genentech, Inc. South San Francisco, CA) en 2 µg/ml y se incubó sobre hielo durante un mínimo de 45 minutos. Las muestras fueron lavadas dos veces con tampón FACS mediante centrifugación. Se añadió una dilución 1:200 de PE antirratón (fragmento IgG Fcy de antirratón de cabra R-ficoeritrina, Jackson Immunoresearch) y se incubó sobre hielo durante 30 minutos. Las muestras volvieron a lavarse dos veces con tampón FACS mediante centrifugación y fueron analizadas por el método FACS.

[0584]. Para los análisis de IgG mediante FACS las muestras fueron preparadas como en el FACS de fagos. Se añadió cada IgG en 5 µg/ml sobre hielo durante 1 hora. Las muestras fueron lavadas dos veces con tampón FACS mediante centrifugación y se añadieron en una dilución de 1:200 de conjugado PE antihumano (fragmento de IgG Fcy antihumano de cabra R-ficoeritrina, Jackson Immunoresearch) durante 30 minutos. Las muestras volvieron a lavarse dos veces con tampón FACS mediante centrifugación y fueron analizadas por el método FACS.

[0585]. *Producción de IgG y determinación de afinidad* – El IgG se purificó con cromatografía de afinidad proteína G. Las determinaciones de afinidad se realizaron mediante análisis Scatchard en células NT LB50 de STEAP-1 293.

Resultados y comentarios

[0586]. *Secuencias de dominio variable murino 120 y diseño de injerto de asignación CDR* – La estructura del aceptor humano usado para la humanización de M2-120.545 se basa en el dominio VL kappa I humano de consenso y en el dominio VH del subgrupo III humano de consenso. Los dominios VL y VH de M2-120.545 murino fueron alineados con los dominios kappa I y subgrupo III humanos; cada región de complementariedad fue identificada e injertada en la estructura del aceptor humano para generar un injerto CDR que pudiese expresarse como un Fab en un fago y se expresase como un IgG. Las secuencias de las regiones variables versión 24 del anticuerpo anti-STEAP-1 humanizado se muestran en las Figuras 2A y 2B. La unión del Fab injerto 120 expresado en fago y del IgG injerto 120 a células que expresan STEAP-1 exógeno (293 STEAP-1 NT LB50) fue sometida a prueba mediante el análisis FACS. Aunque el IgG injerto 120 se unió específicamente a las células que expresan STEAP-1, las señales de

FACS observadas para el IgG injerto 120 fueron menores que las observadas para el IgG 120 quimérico, lo cual indica una pérdida en la afinidad de unión. La expresión de fago del Fab injerto 120 también generó una señal FACS que sólo se observó en las células que expresan STEAP-1. Este cambio fue inferior al observado para el 120 IgG quimérico. El análisis Scatchard del IgG injerto 120 también indicó una pérdida significativa (de aproximadamente 50 veces) en la afinidad de unión (KD = 36 nM para el 120v.78; KD = 260 nM para el injerto 120).

[0587]. ***Humanización de M2-120.545*** – Se han identificado aproximadamente 30 posiciones Vernier que influyen en la conformación CDR y en el empaquetado del dominio VL:VH y cuando se proceda a humanizar anticuerpos deben tenerse en cuenta los cambios en esas posiciones entre las estructuras del donante y las humanas (Foote, J. and Winter, G., J. Mol. Biol.224(2):487-499 (1992)). Una evaluación de la alineación de M2-120.545 murino con el dominio VL kappa I humano de consenso y el dominio VH de consenso del subgrupo III humano reveló diferencias de secuencia en 6 posiciones Vernier clave en el dominio VH: 24, 37, 48, 67, 73, 78 (véase la Figura 2B). Para evaluar la influencia de esas posiciones, se introdujeron individualmente residuos murinos en el dominio VH del subgrupo III de consenso humano del Fab en fago. Esto implicó la realización de las siguientes mutaciones a los Fab injerto 120 expresados en fagos individualmente: A24V (120.v24), V37I (120.v37), V48M (120.v48), F67I (120.v67) y L78F (120.v78). N73T no fue sometido a prueba. Cada variante de fago fue normalizada mediante dilución a un nivel equivalente de expresión de Fab a través de titulación de una señal del epítopo fusionado con la cadena ligera expresada en el fago y después sometida a prueba de evaluación de unión a STEAP-1 mediante análisis de las células que expresan STEAP-1 (293 STEAP-1 NT LB50) y células que no lo expresan (vector 293 S408). El término "2°" se refiere al anticuerpo secundario presente en el análisis FACS. El término "α-120" se refiere al anticuerpo anti-STEAP-1 120 murino. El término "α-10H1" se refiere un anticuerpo de control. Los términos "Fago 24", "Fago 37" y similares se refieren a variantes del anti-STEAP-1 humanizado como se revela en la presente memoria expresadas en fago. "Fago 120 Qu" se refiere a la Quimera 120 expresada en fago y "Fago 120.injerto" se refiere al injerto 120 expresado en fago. (Figura 6). La importancia de la normalización de los clones de fago por su nivel de expresión de Fab se ilustra mediante un análisis FACS del injerto 120 en diferentes tituladores de fagos: $7x10^{12}$ fago/ml en la Figura 6 y 2×10^{11} fago/ml en la Figura 6. Así, la normalización de los diferentes clones de fago por su nivel de expresión fue un paso importante para la evaluación de sus diferencias de afinidad para STEAP-1.

[0588]. De acuerdo con la normalización por niveles de expresión de Fab, la variante del injerto 120 que contiene la mutación adicional A24V (120.v24) produjo un cambio de FACS superior al de otras variantes (Figura 6). Cuando se expresó como un IgG, 120.v24 produjo un cambio de FACS similar al anticuerpo 120 quimérico en todas las concentraciones sometidas a prueba. El ulterior análisis Scatchard de 120.v24 indicó un Kd de 2,2 nM para la unión a células NT LB50 del STEAP-1 293, una mejora del doble respecto del quimera 120 y del M2-120.545 murino original (Tabla 2).

Tabla 2: Afinidad de unión del anticuerpo anti-STEAP-1 con el STEAP-1 de la superficie celular (Kd (nM))

Línea celular	Anti-STEAP-1 murino MAb 120.545 nM	Quimera 120	Anti-STEAP-1 humanizado 120v.24
PC3-PS5.4 (STEAP-1 exógeno)	17,5 nM 187.256 puntos por célula	9,9 nM 103.204 puntos por célula	---
293.LB50 (STEAP-1 exógeno)	4,7 nM 301.100 puntos por célula	4,9 nM 252.892 puntos por célula	2,2 nM 264.172 puntos por célula
LNCaP-BR (STEAP-1 endógeno)	1,5 nM 37.207 puntos por célula	0,9 nM 22.021 puntos por célula	---

[0589]. También se sometió a prueba la actividad de unión de anticuerpos desnudos anti-STEAP-1, murino 120 y quimera 120 usando análsisis FACS. Se comparó la actividad de unión de STEAP-1 exógeno en NT LB50 STEAP-1 estable 293, PS5.4 STEAP-1 estable PC3 y STEAP-1 endógeno en células LNCaP. Los resultados se muestran también en las Figs. 7D-7F. Las células NT LB50 que expresan STEAP-1 humano exógeno en la superficie celular se prepararon transformando de manera estable células 293 (ATCC CRL-1573) con ADN de STEAP-1 humano. Las células PS5.4 que expresan STEAP-1 humano exógeno en la superficie celular se prepararon transformando de manera estable PC3 (ATCC CLL-1435) con ADN de STEAP-1 humano. Las células LNCaP (ATCC CRL-1740) expresan endógenamente a STEAP-1.

Ejemplo 2: Caracterización de anticuerpos anti-STEAP-1

[0590]. Los anticuerpos anti-STEAP-1 (anticuerpos desnudos y conjugados de fármaco con anticuerpo que se describen en la presente memoria) fueron caracterizados o pueden caracterizarse siguiendo métodos estándar.

[0591]. Ensayos basados en el método ELISA: El análisis del anticuerpo anti-STEAP-1 mediante el método ELISA se realizó de la manera siguiente, habiéndose efectuado todas las incubaciones a temperatura ambiente. Las placas de prueba (Nunc Immunoplate) se cubrieron durante dos horas con STEAP-1 purificado en una solución tampón de 50 mm de carbonato de sodio, pH 9,6, después se bloquearon con albúmina de suero bovino al 0,5% en una solución salina tamponada con fosfato (PBS) durante 30 minutos y a continuación se lavó cuatro veces con PBS conteniendo Tween 20 al 0,05% (PBST). Se añadieron supernadantes de anticuerpos de prueba y se incubaron durante dos horas sin dejar de

agitar y después se lavó cuatro veces con PBST. Las placas se desarrollaron añadiendo 100 µl/pocillo de una solución conteniendo 10 mg de dihidrocloruro de fenilendiamina (Sigma, Nº P8287) y 10 µl de una solución de peróxido de hidrógeno al 30% en 25 ml de solución tampón de citrato-fosfato, pH 5,0, e incubando durante 15 minutos. La reacción se interrumpió añadiendo 100 µl/pocillo de ácido sulfúrico 2,5 M. Los datos se obtuvieron leyendo las placas en un lector de placas ELISA automático a una absorbancia de 490 nm.

Caracterización de la unión de anti-STEAP-1 mediante análisis Scatchard:

[0592]. La afinidad de unión del anticuerpo monoclonal puede, por ejemplo, determinarse mediante el análisis Scatchard descrito en Munson y col., Anal. Biochem., 107:220 (1980) usando las técnicas estándar conocidas en la especialidad. Véase también Scathcard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

Ejemplo 3: Producción de conjugados de anticuerpos anti-STEAP-1 con fármacos

[0593]. Producción de ADC auristatinos anti STEAP-1. Los ACD anti-STEAP-1 se produjeron mediante conjugación de anticuerpos anti-STEAP-1 120.545 murino, quimera 120, injerto 120, y variantes de la estructura 120 humanizada con las siguientes fracciones del fármaco ligando: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE, MC-MMAF, vc-MMAE, y vc-MMAF, cuyas fracciones de fármacos y enlazadores y métodos de unión se describen en la presente memoria descriptiva, así como en el documento WO 2004/010957, publicado el 5 de febrero de 2004, en el documento WO2006/034488, publicado el 9 de septiembre de 2005, y en Doronina, S.O. y col., Nature Biotechnol. 21:778-784 (2003), (las referencias de cada documento se incoporan íntegramente en la presente memoria descriptiva a modo de referencia). Antes de la conjugación, los anticuerpos se redujeron parcialmente con TCEP mediante métodos estándares según la metodología descrita en el documento WO 2004/010957. Los anticuerpos parcialmente reducidos se conjugaron con las fracciones de enlazadores de fármacos mencionadas anteriormente mediante métodos estándares según la metodología descrita en Doronina y col., (2003) Nat. Biotechnol. 21:778-784 y patente de EE.UU. N.º 2005/0238649 A1. Los anticuerpos parcialmente reducidos se combinaron con las fracciones del enlazador de fármacos para permitir la conjugación de las fracciones a residuos de cisteína. Las reacciones de la conjugación se absorben y los ADC se purifican. La concentración del fármaco (promedio de fracciones de fármaco por anticuerpo) para cada ADC se determinó mediante HPLC. Tal como se utiliza en la presente memoria descriptiva, el componente ligando-fármaco de un ADC, " –MC-vc-PAB-MMAE" o "-MC-vc-PAB-MMAF" es abreviado, a veces, como " –vcMMAE" o "-vcMMAF", y el componente "-MC-MMAF" es abreviado, a veces, como "MCMMAF" o "mcMMAF".

[0594]. *Producción de conjugados de fármaco con anticuerpos (CFA) maitansinoides anti-STEAP-1* – Los CFA anti-STEAP-1 se produjeron mediante la conjugación de anticuerpos anti-STEAP-1,

murino 120, quimera 120, injerto 120 y variantes de la estructura 120 humanizada con la fracción del fármaco ligando –smcc-DM1. Dicha conjugación puede realizarse de acuerdo con el método descrito en el documento WO 2005/037992 para la conjugación del anticuerpo anti-HER2 Herceptin®.

Ejemplo 4: Ensayo de reducción del volumen del tumor in vivo

[0595]. Se empleó el siguiente protocolo para determinar la eficacia de los anticuerpos anti-STEAP-1 monoclonales no conjugados o conjugados con toxinas para poder reducir el volumen tumoral *in vivo e in vitro.*

[0596]. Líneas celulares de mamíferos y trasplantes de tumores humanos: 293 es una línea celular de riñón embriónico humano inmortalizado (ATCC referencia CRL1573), PC-3 es una línea celular de adenocarcinoma de próstata humano (ATCC referencia CRL1435) y LNCaP es una línea celular de carcinoma de próstata (ATCC CRL1740). Todas las células fueron desarrolladas en un medio Eagle modificado por Dulbecco 50/50 con alto contenido de glucosa, Ham F12 suplementado con suero fetal bovino (SFB) al 10%, glutamina 2 mM, penicilina-estreptomicina al 1% y cultivado a 37°C en CO_2 al 5%. Las líneas celulares estables 293 y PC-3 se generaron mediante transfección (Fugene, Roche) con un vector dirigido por citomegalovirus que codifica a STEAP-1 de longitud completa (LB50 y PS5.4, respectivamente) o a un vector vacío y seleccionado en 400 µg/ml G418 (Geneticin, Life Technologies). Los modelos de explantes de próstata humana, LuCAP 77 y LuCAP 35V, fueron facilitados por la Universidad de Seattle.

[0597]. La expresión del STEAP-1 exógeno y endógeno en la superficie celular se demostró mediante inmunohistoquímica (IHQ) y análisis FACS de la manera siguiente. Los anticuerpos anti-STEAP-1 de oveja y ratón (Agensys, Inc., Santa Monica, CA) se generaron contra un péptido aminoterminal intracelular de STEAP-1 (véase Hubert, R.S., Vivanco, I. y col., PNAS 25:14523-14528 (1999)). Los anticuerpos monoclonales contra los dominios extracelulares de STEAP-1 (Agensys Inc.) se generaron mediante inmunización de ratones con células 293T transfectadas transitoriamente con STEAP-1. En el análisis IHQ, para la detección se usó el anticuerpo anti-STEAP-1 primario de oveja. Para el análisis FACS, se desarrollaron las células a una confluencia del 90% y se sacaron de las placas usando EDTA 2 mM en PBS. Las células se lavaron y se volvieron a suspender en una solución tampón FACS (PBS con BSA al 1%) y se incubaron durante 60 minutos con anticuerpos anti-STEAP-1 a temperatura ambiente seguidos por 60 minutos con el anticuerpo secundario apropiado conjugado con ficoeritrina. Se llevaron a cabo análisis en FACSscan (BD Biosciences). Para la inmunofluorescencia, las células se desarrollaron en cámaras de cultivo durante toda la noche y después de incubaron con anticuerpo primario a 37°C durante 60 minutos. Las células se fijaron en paraformaldehído, se bloquearon en BSA al 1% y se incubaron con el anticuerpo secundario apropiado conjugado con fluoresceína.

[0598]. Se usaron modelos xenotrasplantados de cáncer de próstata *in vivo* para probar la eficacia de los ADC (conjugados fármaco y anticuerpo) anti-STEAP-1. Esos modelos incluyeron líneas celulares humanas LNCaP (ATCC CRL-1740 o Southern Research Institute, Birmingham, AL). Los modelos de explante de próstata incluyeron LuCaP 77 y LuCaP35V (Universidad de Washington, Seattle, WA). Cada modelo de explante de próstata se mantuvo mediante trasplante seriado en ratones beige SCID machos castrados (modelo andrógeno independiente, LuCAP 35V) o no castrados (modelo andrógeno dependiente, LuCAP 77) de Charles River Lab. Los ratones no castrados recibieron un granulado de testosterona antes de la implantación, mientras que la castración se efectuó al menos dos semanas antes de la implantación del tumor para impedir que los niveles de testosterona llegasen a su punto más bajo. Cuando los ratones donantes tuvieron tumores de entre 800-1.000 mm^3, se extirpó asépticamente el tejido tumoral y se procedió a diseccionarlo en trozos pequeños (aproximadamente 20 mm^3) aptos para el trasplante a los animales de estudio. Se colocó el tumor dentro de una bolsa en el lugar de implantación y se emplearon grapas para cerrar la piel. Para el modelo de línea celular LNCaP, las células LNCaP desarrolladas *in vitro* se inyectaron subcutáneamente a razón de 8-10 millones de células por ratón en Matrigel al 50% en ratones beige SCID machos que habían recibido un granulado de testosterona. Cuando el tamaño medio del tumor llegó a 100-200 mm^3, los animales fueron distribuidos aleatoriamente en diez grupos de diez ratones cada uno y recibieron una única administración IV del ADC anticuerpo de prueba o del anticuerpo de control (desnudo o control). En algunos experimentos, se administraron dosis múltiples de anticuerpo de prueba o de control (véanse las Figuras 8A, 9 y 10). En algunos experimentos, se administró una dosis única del anticuerpo de prueba y de control, como puede verse en las Figuras 8B y 11. Donde el modelo de explante de próstata fue LuCap 77, se implantó en el ratón un granulado de testosterona aproximadamente 3-7 días antes del trasplante del tumor exógeno. Los tumores se midieron dos veces por semana durante 4 semanas y después una o dos veces por semana durante el tiempo restante del estudio, o una vez por semana a lo largo de todo el estudio. La reducción significativa del tamaño del tumor en los animales de prueba con el transcurso del tiempo se consideró una indicación de eficacia. En algunos casos, el volumen del tumor disminuyó significativamente en relación con el tamaño inicial y esa reducción se mantuvo a lo largo del estudio. Los resultados se representan gráficamente en las Figuras 8-11.

Los conjugados del fármaco auristatina anti-STEAP-1 reducen el volumen del tumor de próstata *in vivo*

[0599]. La administración de 120-MC-vc-PAB-MMAE anti-STEAP murino en 3 mg/kg fue eficaz en un modelo xenotrasplantado (células LNCaP-Ner) de tumor de próstata. Como controles se usaron PBS y anti-gp120-MC-vc-PAB-MMAE (3 mg/kg). Las dosis se administraron en los días 0, 7 y 14. Véase la Figura 8A.

[0600]. La administración de anticuerpo 120v.24-MC-vc-PAB-MMAE (3 mg/kg), 120v.24-MC-MMAF (6 mg/kg), 120v.24-MC-MMAF (12 mg/kg) anti-STEAP-1 humanizado, y de 120 MC-vc-PAB-MMAE (3 mg/kg) quimérico anti-STEAP-1 a ratones beige SCID trasplantados con tumor LNCap-Ner (tratados con un granulado de testosterona como se ha descrito en la presente memoria) demostró resultar eficaz. Como controles se usaron vehículo, MC-vc-PAB-MMAE anti-artemisa (3 mg/kg) y MC-MMAF anti-artemisa (12 mg/kg). Las dosis se administraron en los días indicados en la Figura 8. Los resultados se grafican en la Figura 8B.

[0601]. La administración de anticuerpo 120 MC-vc-PAB-MMAE (3 mg/kg) quimérico anti-STEAP-1 y 120 MC-MMAF (6 mg/kg) quimérico anti-STEAP-1 demostró resultar eficaz en un modelo xenotrasplantado de cáncer de próstata de ratones beige SCID trasplantados con células LNCaP. Se administraron a los ratones tres dosis aproximadamente en los días 15, 25 y 30 de 3 mg/kg (vcMMAE anti-STEAP) o 6 mg/kg (mcMMAF anti-STEAP). Como controles se usaron MC-vc-PAB-MMAE anti-artemisa (3 mg/kg) y MC-MMAF anti-artemisa (6 mg/kg). Véase la Figura 9.

[0602]. La administración del anticuerpo 120 MC-vc-PAB-MMAE quimérico anti-STEAP-1 (3 mg/kg) resultó eficaz en un modelo xenotrasplantado de cáncer de próstata de ratón macho beige SCID (andrógeno dependiente) trasplantado con células LuCap 77. Los controles fueron vehículos y MC-vc-PAB-MMAE anti-artemisa. Se administraron tres dosis en 3 mg/kg de anticuerpos de prueba y control. Véase la Figura 10.

[0603]. La administración del anticuerpo 120v.24-MC-vc-PAB-MMAE humanizado anti-STEAP-1 (3 mg/kg) y del anticuerpo 120v.24-MC-MMAF anti-STEAP-1 (6 mg/kg y 12 mg/kg) a ratón beige SCID castrado trasplantado con tumor de próstata LuCap35V resultó eficaz en relación con los controles. La concentración de fármaco fue de 3.1 por anticuerpo. Se administraron anticuerpos de control MC-MMAF anti-artemisa en 12 mg/kg y 120-MC-vc-PAB-MMAE anti-gp en 6 mg/kg. Véase la Figura 11.

Los conjugados del fármaco auristatina anti-STEAP-1 reducen el volumen del tumor de próstata *in vitro*

[0604]. Se realizaron ensayos de destrucción de células *in vitro* para evaluar la eficacia de los conjugados de fármaco y anti-STEAP-1 para la inhibición del desarrollo y/o la destrucción de las células con expresión de STEAP-1. En síntesis, las células con expresión de STEAP-1 fueron colocadas en placas a razón de aproximadamente 2.000 células/pocillo en una placa de 96 pocillos y tratadas 24 horas más tarde por duplicado con conjugado de fármaco y anticuerpo. Las placas fueron incubadas durante 5-7 días a 37°C y desarrolladas con el kit de ensayo luminiscente de viabilidad celular CellTiter-Glo® (Promega, Madison, WI, EE.UU.). Las células de prueba incluyeron células PS5.4 (células PC3 con expresión de STEAP-1 exógeno), LB50 (células 293 con expresión de STEAP-1 exógeno), células PC3 transfectadas con un solo vector, células 293 transfectadas con un solo vector y células LNCaP con expresión de STEAP-1 endógeno. Los conjugados de fármaco y anticuerpo sometidos a prueba incluyeron anticuerpo de control MC-MMAF,

anticuerpo de control vc-MMAE, anticuerpo 120 vc-MMAE quimérico anti-STEAP-1, anticuerpo 120 MC-MMAF quimérico anti-STEAP-1 (dos lotes diferentes de material) y anticuerpo vc-MMAF quimérico anti-STEAP-1. Los resultados se muestran en las Figuras 14A-E.

Ejemplo 5: Preparación de anticuerpos anti-STEAP-1 diseñados con cisteína para conjugación mediante reducción y reoxidación.

[0605]. Los anticuerpos monoclonales anti-STEAP-1 diseñados con cisteína de longitud completa (ThioMabs) expresados en las células CHO se disuelven en 500 mM de borato de sodio y 500 mM de cloruro de sodio a pH 8,0 aproximadamente y se reducen con un exceso de TCEP de 1 mM equivalente a entre 50 y 100 veces (tris(2-carboxietil)fosfino hidrocloruro, Getz y col., (1999) *Anal. Biochem.* Vol 273:73-80; Soltec Ventures, Beverly, MA) durante 1 ó 2 horas a 37 °C. El ThioMab reducido se diluye y se carga en la columna HiTrap S en acetato sódico 10 mM, pH 5, y se eluye con PBS que contiene cloruro de sodio 0,3 M. El ThioMab reducido eluido se trata con 2 mM de ácido dehidroascórbico (dhAA) a pH 7 durante 3 horas o 2 mM de sulfato de cobre acuoso ($CuSO_4$) a temperatura ambiente durante la noche. La oxidación al aire del ambiente también puede ser eficaz. El tampón se cambia por medio de la elusión con la resina Sephadex G25 y se eluye con PBS con un 1 mM de DTPA. Se controla el valor tiol/Ac mediante la determinación de la reducción de la concentración de anticuerpos a partir de la absorbancia a 280 nm de la solución y la concentración de tiol mediante la reacción con DTNB (Aldrich, Milwaukee, WI) y la determinación de la absorbancia a 412 nm.

Ejemplo 6: Preparación de conjugados de fármaco y anticuerpo anti-STEAP-1 diseñados con cisteína mediante conjugación de anticuerpos anti-STEAP-1 diseñados con cisteína y ligando-fármaco intermedios

[0606]. Después de los procedimientos de reducción y reoxidación del Ejemplo 5, el anticuerpo anti-STEAP-1 diseñado con cisteína se disuelve en un tampón de PBS (solución salina fosfatada) y se enfría en hielo. Aproximadamente, 1,5 equivalentes molares con respecto a las cisteínas diseñadas por anticuerpo de un enlazador intermedio a fármaco auristatina, como MC-MMAE (maleimidocaproilo-monometilo auristatina E), MC-MMAF, MC-val-cit-PAB-MMAE o MC-val-cit-PAB-MMAF, con un grupo fucional de reactivos de tiol, como la maleimida, se disuelve en DMSO, diluye en acetonitrilo y agua, y añade al anticuerpo reoxidado, reducido y enfriado en PBS. Después de aproximadamente una hora, se agrega un exceso de maleimido para extinguir la reacción y cubrir cualquier anticuerpo del grupo tiol no reactivo. La mezcla de reacción se concentra mediante ultrafiltración centrífuga y el anticuerpo anti-STEAP-1 conjugado con fármacos y diseñado con cisteína se purifica y se desala por elusión a través de la resina G25 en PBS, se filtra a través de filtros de 0,2 µm en condiciones de esterilización y se congela para almacenarse.

[0607]. Mediante al procedimiento indicado anteriormente, se prepararon los siguientes conjugados de fármaco y anticuerpo anti-STEAP-1 diseñados con cisteína (donde la numeración de las variantes es la numeración de Kabat estandarizada para la cadena ligera y la numeración de la UE para la cadena pesada), como se indica en la presente memoria y en la Figura 17):

120-MC-MMAF humano tio meidante conjugación de la cadena ligera V205C tio-HC-10F4v3 hu 120 y MC-MMAF;

120-MC-MMAF humano tio por conjugación de la cadena pesada A118C tio hu 120 y MC-MMAF

120-MC-val-cit-PAB-MMAE humano tio por conjugación de cadena ligera V205C tio hu 120 y MC-val-cit-PAB-MMAE; y

120-MC-val-cit-PAB-MMAE humano tio por conjugación de cadena pesada A118C tio hu 120 y MC-val-cit-PAB-MMAE.

Ejemplo 7: Caracterización de los anticuerpos anti-STEAP-1 diseñados con cisteína

[0608]. Los conjugados de fármaco y anticuerpo anti-STEAP-1 diseñados con cisteína (TDC) preparados como se ha descrito anteriormente fueron sometidos a ensayo para confirmar que mantienen la actividad del anticuerpo progenitor *in vitro*. Se valoraron los TDC anti-STEAP-1 120-vc-PAB-MMAE (LCV205C) humano tio (abreviado como huSteap1 TDC (L205C) vcE y 120-vc-PAB-MMAE (HCA118C) humano tio (abreviado como huSteap1 TDC (HCA118C) vcE para comprobar la unión a STEAP-1 mediante análisis FACS en células con expresión de STEAP-1 (293 STEAP-1 NT LB50) y células sin expresión de STEAP-1 (293 vector S408). El término "sólo 2°" se refiere al anticuerpo secundario presente en el análisis FACS. Los controles TDC (vcE) y std ADC (vcE) son conjugados de fármaco y anticuerpos de control vc-PAB-MMAE tio y no tio, respectivamente. El ADC (std) huSteap-1 es un conjugado de fármaco y vc-PAB-MMAE derivado del anticuerpo progenitor humano anti-STEAP-1. Como queda demostrado, los TDC produjeron cambios FACS similares a los del ADC huSteap-1 progenitor.

[0609]. También se realizaron ensayos de destrucción de células *in vitro* para evaluar la eficacia de los conjugados de fármaco y anticuerpo anti-STEAP-1 para la inhibición del desarrollo y/o la destrucción de las células con expresión de STEAP-1. En síntesis, las células con expresión de STEAP-1 fueron colocadas en placas a razón de aproximadamente 2.000 células/pocillo en una placa de 96 pocillos y tratadas 24 horas más tarde por duplicado con conjugado de fármaco y anticuerpo. Las placas fueron incubadas durante 5-7 días a 37°C y desarrolladas con el kit de ensayo luminiscente de viabilidad celular CellTiter-Glo® (Promega, Madison, WI, EE.UU.). Las células de prueba incluyeron PS5.4 (células PC3 con STEAP-1 de expresión exógena), LB50 (células 293 con STEAP-1 de expresión exógena y células LNCaP con STEAP-1 de expresión endógena. Los conjugados de fármaco y anticuerpo sometidos a prueba incluyeron anticuerpo de control vc-MMAE (control std ADC (vcE)), anticuerpo tio de control vc-MMAE

(control TDC (vcE)), los TDC anti-STEAP-1 humano tio 120-vc-PAB-MMAE (LCV205C) (abreviado como huSteap1 TDC (L205C) vcE y humano tio 120-vc-PAB-MMAE (HCA118C) (abreviado como huSteap1 TDC (HCA118C) vcE), y huSteap1 ADC (std), un conjugado de fármaco y vc-PAB-MMAE derivado del anticuerpo de progenitor humano anti-STEAP-1. Como se muestra en las Figuras 19A-C, los TCD anti-STEAP-1 mantienen la actividad del ADC progenitor *in vitro*.

Ejemplo 8: Ensayos de reducción del volumen del tumor *in vivo* para conjugados de fármaco y anticuerpo anti-STEAP-1 diseñados con cisteína

[0610]. Se usaron modelos xenotrasplantados de cáncer de próstata *in vivo* para probar la eficacia de los ADC (conjugados fármaco y anticuerpo) anti-STEAP-1 diseñados con cisteína. Estos modelos y los protocolos de ensayo empleados corresponden a los descritos en el Ejemplo 4.

[0611]. La administración del TDC 120-vc-PAB-MMAE (HCA118C) (abreviado como huSteap1 HC TDC vcE) humano tio anti-STEAP-1 (3 mg/kg) a ratones beige SCID trasplantados con tumor LNCap-Ner (tratados con un granulado de testosterona como se ha descrito en la presente memoria) demostró resultar eficaz. Como controles se emplearon vehículo (PBS), anticuerpo de control vc-MMAE (ADC std ctrl vcE) y anticuerpo de control tio vc-MMAE (TDC HC ctrl vcE). También se comparó el efecto del TDC anti-STEAP-1 con el anticuerpo 120-MC-vc-PAB-MMAE (hu Steap1 std ADC vcE) anti-STEAP-1 como un control positivo. Se administró una dosis única en el día 0. Todos los anticuerpos se administraron en 3 mg/kg. Los resultados se representan gráficamente en la Figura 20.

[0612]. La Figura 21 muestra que la administración de TDC anti-STEAP-1 120-vc-PAB-MMAE humano tio (HCA118C) (abreviado como hu Steap 1 HC TDC vcE) en 3 mg/kg y de TDC anti-STEAP-1 120-MC-MMAF humano tio (HCA118C) (abreviado como hu Steap1 HC TDC mcF) en 3 ó 6 mg/kg resultó eficaz en un modelo xenotrasplantado de cáncer de próstata de ratón beige SCID trasplantado con células LNCaP. Se administraron a los ratones dosis únicas en el día 0 en 0,3, 1 ó 3 mg/kg (huSteap1 HC TDC vcE) o 1, 3 ó 6 mg/kg (huSteap1 HC TDC mcF). Como controles se emplearon vehículo (PBS), anticuerpo de control vc-MMAE (ADC std ctrl vcE) y anticuerpo de control tio vc-MMAE (TDC HC ctrl vcE).

[0613]. La Figura 22 muestra que la administración de TDC anti-STEAP-1 120-vc-PAB-MMAE humano tio (HCA118C) (abreviado como hu Steap 1 HC TDC vcE) en 3 mg/kg y de TDC anti-STEAP-1 120-MC-MMAF humano tio (HCA118C) (abreviado como hu Steap1 HC TDC mcF) en 3 ó 6 mg/kg resultó eficaz en un modelo xenotrasplantado de cáncer de próstata de ratón macho beige SCID (andrógeno dependiente) trasplantado con células LuCap 35V. Se administraron a los ratones dosis únicas en el día 0 en 0,3, 1 ó 3 mg/kg (huSteap1 HC TDC vcE) o 1, 3 ó 6 mg/kg (huSteap1 HC TDC mcF). Como controles se emplearon vehículo (PBS), anticuerpo de control vc-MMAE (ADC std ctrl vcE) y anticuerpo de control tio vc-MMAE (TDC HC ctrl vcE).

Ejemplo 9: Preparación y Caracterización de Anticuerpo SGIV anti-STEAP-1 a partir de Anticuerpo 120 variante 24

[0614]. Se preparó otra variante de anticuerpo LC anti-STEAP-1, en donde la cadena ligera y regiones de estructura, son además, modificadas para obtener niveles mejorados de expresión de anticuerpo.

Materiales y Métodos

[0615]. Los números residuales de conformidad con Kabat ((Kabat *et al., Sequences of proteins of immunological interest*, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Se usaron abreviaturas de aminoácido de una sola letra. Las degeneraciones de ADN son representadas usando el código IUB (N = A/C/G/T, D = A/G/T, V = A/C/G, B= C/G/T, H= A/C/T, K = G/T, M = A/C, R = A/G, S = G/C, W= A/T, Y = C/T).

[0616]. *Preparación de una Variante de Cadena Ligera Revisada*: Se generó y caracterizó una variante del anticuerpo 120v.24, designada "Simmons IV", o simplemente "SGIV". La secuencia de aminoácido de la cadena ligera SGIV se proporciona en la SEC ID NO:90. Esta secuencia, alineada con las regiones correspondientes del anticuerpo mu 120 (SEC ID NO:5) y el anticuerpo 120.v24 (SEC ID NO:91), se muestra en la Figura 23.

[0617]. ***Valoración de variante SGIV comparada con la variante 120.v24*** - Los anticuerpos SGIV y 120.v24, expresados como IgG, fueron expresados por análisis FACS usando las líneas de células positivas Steap1 establemente transformadas 293 Steap1 NT LB48, 293 Steap1 NT LB50, y 293 Steap1 NT LB53, así como también en células LNCaP, las cuales expresan STEAP-1 endógenas (Figura 28). Las células se prepararon como se describe en el Ejemplo 1. Cada IgG se agregó a 5 µg/ml en hielo por horas. Las muestras se lavaron dos veces con amortiguador FACS por centrifugación y se agregó por 30 minutos, una dilución 1:200 de conjugado PE anti-humano (Fragmento IgG Fcy anti-humano de cabra de R-ficoeritrina, Jackson Immunoresearch). Las muestras fueron nuevamente lavadas dos veces con amortiguador FACS por centrifugación y las muestras fueron analizadas por FACS.

[0618]. *Determinación de Afinidad a base de Scatchard de SGIV y 120.v24 que se enlaza a STEAP-1* – Las afinidades de los anticuerpos 120.v24 y Simmons IV ("SGIV") a STEAP-1, se determinaron usando el análisis Scatchard de conformidad con los métodos estándares. El IgG se purificó con cromatografía de afinidad de Proteína G. Las determinaciones de afinidad se realizaron por análisis Scatchard en células PC-3-PS5.4, 293-LB50 y LNCap-BR por duplicado. Los trazos Scatchard de 120.v24 y SGIV en células LNCaP BR y células 293.LB50 se muestran en las Figuras y 26, respectivamente. Se muestra en la Figura 27, una tabla que compara las afinidades de enlace promedio para mu 1789, mu 120,

quimera Fc, 120.v24 humanizado, tio-120.v24 y tio-SGIV en células 293-LB50 y LNCaP-BR, así como también en células 293 que expresan temporalmente STEAP-1-.

[0619]. *Mutagénesis Dirigida al Sitio de SGIV y 120.v24:* Variantes de anticuerpos SGIV y 120.v24 se prepararon usando protocolos de mutagénesis estándar como se describe anteriormente. La primera clase de variantes que resultan de la mutagénesis dirigida al sitio por medio de residuos particulares de Simmons IV ("SGIV"), fueron reemplazadas con el residuo correspondiente de 120.v24 para mejorar además, la afinidad de enlace. Las variantes específicas producidas, como se muestra en la Figura 24, fueron como sigue:

(1) LS.VLVH1, en donde los residuos 42 ("Q") y 43 ("P") fueron modificados a "K" y "A", respectivamente (SEC ID NO: 92).

(2) LS.VLVH2, en donde el residuo 3 ("V") se modificó a "Q", los residuos 42 ("Q") y 43 ("P"), se modificaron a "K" y "A", respectivamente, y el residuo 85 ("V"), se modificó a "T" (SEC ID NO:93).

(3) LS.Q, en donde el residuo 3 ("V") se modificó a "Q") (SEC ID NO:94).

(4) LS.CH1, en donde el residuo 15 ("L") se modificó a "V" y el residuo 83 ("V") se modificó a "F" (SEC ID NO:95).

[0620]. Se generó una segunda clase de variantes a través de mutagénesis dirigida al sitio, en donde residuos particulares de 120.v24, fueron reemplazados con el residuo correspondiente de Simmons IV (SIGV), en un intento por mejorar los niveles de expresión de anticuerpo. Las variantes específicas, como se muestra en la Figura 24, fueron como sigue:

(1) ED.FW1, en donde el residuo 3 ("Q") se modificó a "V"; el residuo 9 ("S") se modificó a "D"; el residuo 12 ("S") se modificó a "A"; el residuo 13 ("A") se modificó a "V"); el residuo 15 ("V") se modificó a "L"; el residuo 17 ("D") se modificó a "E"; el residuo 19 ("V") se modificó a "A"; y el residuo 22 ("T") se modificó a "N" (SEC ID NO:96).

(2) ED.FW2, en donde los residuos 42 ("K") y 43 ("A") de 120.v24, fueron modificados a "Q" y "P", respectivamente (SEC ID NO:97).

(3) ED.FW3, en donde el residuo 60 ("S") se modificó a "D"; el residuo 80 ("P") se modificó a "A"; el residuo 83 ("F") se modificó a "V"; y el residuo 85 ("T") se modificó a "V" (SEC ID NO:98).

(4) ED.all, en donde el residuo 3 ("Q") se modificó a "V"; el residuo 9 ("S") se modificó a "D"; el residuo 12 ("S") se modificó a "A"; el residuo 13 ("A") se modificó a "V"; el residuo 15 ("V") se

modificó a "L"; el residuo 17 ("D") se modificó a "E"; el residuo 19 ("V") se modificó a "A"; el residuo 22 ("T") se modificó a "N"; los residuos 42 ("K") y 43 ("A") de 120.v24, fueron modificados a "Q" y "P"; el residuo 60 ("S") se modificó a "D"; el residuo 80 ("P") se modificó a "A"; el residuo 83 ("F") se modificó a "V"; y el residuo 85 ("T") se modificó a "V" (SEC ID NO:99).

(5) ED.Pro, en donde el residuo 43 ("A") se modificó a "P" y el residuo 80 ("P") se modificó a "A" (SEC ID NO:100) .

(6) ED.pl, en donde el residuo 9 ("S") se modificó a "D"; el residuo 42 ("K") se modificó a "Q" y el residuo 60 ("S") se modificó a "D" (SEC ID NO:101).

Resultados y Discusión

[0621]. *Preparación de anticuerpo SGIV* – Las secuencias de la región variable versión 24 (120.v24) de anticuerpo anti-STEAP-1, se muestran en las Figuras 23 y2 4 (SEC ID NO:91). Usando mutagénesis dirigida al sitio, se prepararon otras variantes llamadas "Simmons IV" o simplemente "SGIV", usando protocolos de mutagénesis estándar, como se describe anteriormente. Las Figuras 23 y 24 muestran la secuencia de cadena ligera SGIV en alineamiento con aquella del anticuerpo mu 120 y 120.v24. Los tituladores de varias colectas de anticuerpo SGIV, se muestran en la Figura 29.

[0622]. *Comparación de Enlace de SGIV y 120.v24 a STEAP-1 usando FACs* – La capacidad de ambos anticuerpos, 120.v24 y SGIV, para enlazarse a STEAP-1 expresado en la superficie celular, fue medida usando FACS. El enlace de anticuerpo a las líneas celulares que expresan ya sea STEAP-1 exógeno (293 STEAP-1 LB48, 293 STEAP-1 LB50 y 293 STEAP-1 LB53) o STEAP-1 endógeno (LNCaP.Br), fueron medidas por duplicado; los resultados se resumen en la Figura 28. Como se muestra en la Figura 28, ambos anticuerpos fueron capaces de enlazarse a STEAP-1 en las cuatro líneas celulares.

[0623]. *Afinidad de enlace al anticuerpo SGIV a STEAP-1 y Comparación con 120.v24* – Las afinidades de enlace del SGIV y 120.v24 a STEAP-1, fueron examinadas usando análisis Scatchard. Los trazos Scatchard de 120.v24 y SGIV en células LNCaP BR y células 293.LB 50, se muestran en las Figuras 25 y 26, respectivamente. Una tabla que compara las afinidades de enlace promedio, para mu 1789, mu 120, quimera Fc, anticuerpos humanizados 120.v24, tio-120.v24 y tio-SGIV en células PC-3-PS5.4, 293-LB50 y LNCaP-BR, así como también en células 293 que expresan temporalmente STEAP-1, se muestra en la Figura 27. Los resultados indican que la afinidad de enlace del anticuerpo 120.v24 en células 293-LB50 y LNCaP.BR, es bruscamente 1.5 veces aquella de la variante SGIV.

[0624]. Aunque la anterior invención se ha descrito en detalle a modo de ejemplo a efectos de claridad y comprensión, las descripciones y ejemplos no se deben interpretar de forma que limiten el ámbito de la invención. El contenido de todas las patentes y documentos científicos citados en la presente memoria descriptiva quedan expresamente incorporados en la misma en su integridad a modo de referencia.

LAS REIVINDICACIONES SON:

1. Un anticuerpo humanizado monoclonal que se une a STEAP-1, donde el anticuerpo comprende una cadena pesada (HC) que comprende:

(1) una HVR-H1 que contiene la secuencia de aminoácidos de SEC ID NO:14;

(2) una HVR-H2 que contiene la secuencia de aminoácidos de SEC ID NO:15;

(3) una HVR-H3 que contiene la secuencia de aminoácidos de SEC ID NO:16; y

(4) una HC-FR1 que contiene la secuencia de aminoácidos de SEC ID NO:25.

2. El anticuerpo de la reivindicación 1 que comprende además, al menos una, dos o tres regiones de estructuras (FRs) HC seleccionadas de:

(1) una HC-FR2 que contiene la secuencia de aminoácidos de SEC ID NO:22, 75, o 76;

(2) una HC-FR3 que contiene la secuencia de aminoácidos de SEC ID NO:23, 78, o 79; y

(3) una HC-FR4 que contiene la secuencia de aminoácidos de SEC ID NO:24.

3. El anticuerpo de la reivindicación 1, que comprende además una cadena ligera (LC) que comprende:

(1) una HVR-L1 que contiene la secuencia de aminoácidos de SEC ID NO:11;

(2) una HVR-L2 que contiene la secuencia de aminoácidos de SEC ID NO:12; y

(3) una HVR-L3 que contiene la secuencia de aminoácidos de SEC ID NO:13.

4. El anticuerpo de la reivindicación 3, que comprende una cadena ligera (LC) de SEC ID NO:6.

5. El anticuerpo de la reivindicación 1, donde la HC comprende la SEC ID NO:10.

6. El anticuerpo de la reivindicación 4, donde la HC comprende la SEC ID NO:10.

7. El anticuerpo de la reivindicación 1, donde el anticuerpo es un fragmento de anticuerpo seleccionado entre el fragmento Fab, Fab'-SH, Fv, scFv, o (Fab')2.

8. Un método de detección de la presencia de STEAP-1 en una muestra biológica, el método comprende contactar la muestra biológica con un anticuerpo de la reivindicación 1 en condiciones que faciliten la unión del anticuerpo a STEAP-1, y detectar si un complejo está formado entre el anticuerpo y STEAP-1.

9. El método de la reivindicación 8, donde la muestra biológica se obtiene de un paciente que se cree que padece un trastorno proliferativo de células de próstata, pulmón, colon, vejiga, ovario o sarcoma de Ewing.

10. Un inmunoconjugado que comprende un anticuerpo de la reivindicación 1 que se une covalentemente a un agente citotóxico.

11. El inmunoconjugado de la reivindicación 10, donde el agente citotóxico se selecciona a partir de una toxina, un agente quimioterapéutico, una fracción de fármaco, un antibiótico, un isotopo radioactivo y una enzima nucleolítica.

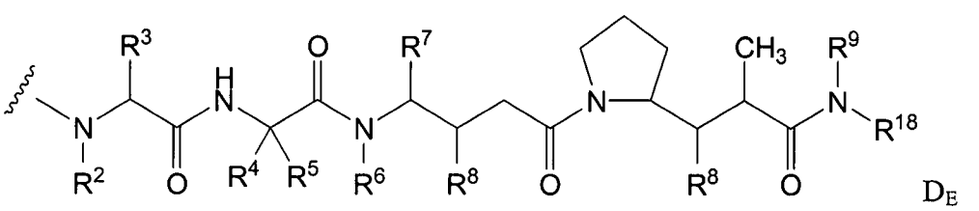
12. Un inmunoconjugado de la reivindicación 11, donde el inmunoconjugado tiene la fórmula Ab-(L-D)p, y donde:
(a) Ab es el anticuerpo de la reivindicación 1 o la reivindicación 3;
(b) L es un enlazador;
(c) D es una fracción de fármaco.

13. El inmunoconjugado de la reivindicación 12, en el que L se selecciona a partir de 6-maleimidocaproilo (MC), maleimidopropanoilo (MP), valina-citrulina (val-cit), alanina-fenilalanina (ala-phe), p-aminobenciloxicarbonil (PAB), N-Succinimidil

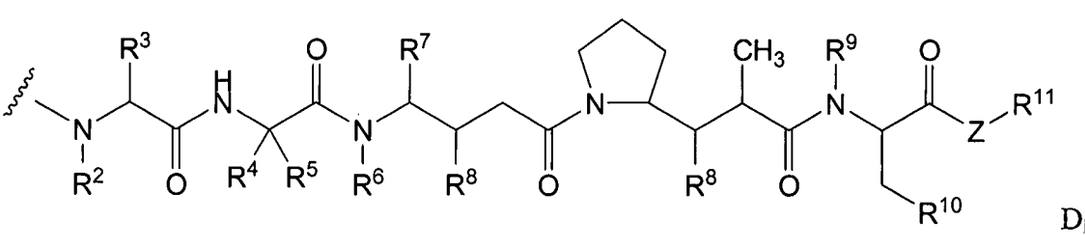
4-(2-piridiltio) pentanoato (SPP), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato (SMCC), N-Succinimidil (4-yodo-acetil) aminobenzoato (SIAB), y 6-maleimidocaproilo-valina-citrulina-p-aminobenciloxicarbonil (MC-vc-PAB).

14. El inmunoconjugado de la reivindicación 12, en el que D se selecciona a partir de una auristatina y una dolastatina.

15. El inmunoconjugado de la reivindicación 14, en el que D es una fracción de fármaco de la fórmula D_E o D_F:



D_E



D_F

y donde R^2 y R^6 son, cada uno, metilo; R^3 y R^4 son cada uno isopropilo; R^7 es sec butilo, cada R^8 se selecciona independientemente entre CH_3, O-CH_3, OH, y H; R^9 es H; R^{10} es arilo; Z es –O– o –NH–; R^{11} es H, C_1-C_8 alquilo o –$(CH_2)_2$–O–$(CH_2)_2$–O–$(CH_2)_2$–O–CH_3; y R^{18} es –$C(R^8)_2$–$C(R^8)_2$–arilo; y
(d) p oscila entre aproximadamente 1 y 8.

16. El inmunoconjugado de la reivindicación 12, con actividad de destrucción celular in vitro o in vivo 36.

17. El inmunoconjugado de la reivindicación 12 donde el inmunoconjugado tiene la fórmula Ab-(L-MMAE)p,
donde L es un enlazador y p oscila entre 2 y 5.

18. El inmunoconjugado de la reivindicación 17, donde L comprende val-cit.

19. El inmunoconjugado de la reivindicación 17, donde L comprende MC.

20. El inmunoconjugado de la reivindicación 17, donde L comprende PAB.

21. El inmunoconjugado de la reivindicación 17, donde L comprende MC-vc-PAB.

22. El inmunoconjugado de la reivindicación 12, donde D es un maitansinoide.

23. El inmunoconjugado de la reivindicación 22, donde D se selecciona a partir de DM1, DM3 y DM4.

24. El inmunoconjugado de la reivindicación 22, donde el enlazador está unido al anticuerpo a través de un grupo tiol en el anticuerpo.

25. El inmunoconjugado de la reivindicación 22, donde el enlazador L se selecciona a partir de N-Succinimidil 4-(2-piridiltio) pentanoato (SPP), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato (SMCC) y N-Succinimidil (4-yodo-acetil) aminobenzoato (SIAB).

26. El inmunoconjugado de la reivindicación 25, donde D es DM4.

27. El inmunoconjugado de la reivindicación 26, donde L comprende un SPP.

28. El inmunoconjugado de la reivindicación 26, donde L comprende SMCC.

29. El inmunoconjugado de la reivindicación 26, donde p oscila entre 2 y 6.

30. Una composición farmacéutica que contiene el inmunoconjugado de la reivindicación 12 y un vehículo farmacéuticamente aceptable.

31. Un método de tratamiento de un trastorno proliferativo de células que comprende la administración a un sujeto de una cantidad eficaz de la composición farmacéutica de la reivindicación 30.

32. El método de la reivindicación 31, en el que el trastorno proliferativo de células se selecciona a partir de de un trastorno proliferativo de células de próstata, pulmón, colon, vejiga, ovario y sarcoma de Ewing.

33. Un anticuerpo diseñado con cisteína que comprende el anticuerpo de la reivindicación 1 o reivindicación 3 donde uno o más residuos de aminoácidos son sustituidos con uno o más aminoácidos de cisteína libre con un valor de reactividad del tiol que oscila entre 0,6 y 1,0.

34. El anticuerpo diseñado con cisteína de la reivindicación 33, en el que uno o más residuos de aminoácidos de cisteína libre se encuentran en una cadena ligera.

35. El anticuerpo diseñado con cisteína de la reivindicación 33, en el que uno o más residuos de aminoácidos de cisteína libre se encuentran en una cadena pesada.

36. Un inmunoconjugado que comprende el anticuerpo diseñado con cisteína de la reivindicación 33, que se une covalentemente a un agente citotóxico.

37. El inmunoconjugado de la reivindicación 36, donde el agente citotóxico se selecciona a partir de una toxina, un agente quimioterapéutico, una fracción de fármaco, un antibiótico, un isotopo radioactivo y una enzima nucleolítica.

38. El anticuerpo diseñado con cisteína de la reivindicación 33, donde el anticuerpo se une covalentemente a una marca de captura, una marca de detección o un soporte sólido.

39. Un conjugado de anticuerpo con fármacos que comprende el anticuerpo diseñado con cisteína de la reivindicación 33, donde el anticuerpo se une covalentemente a una fracción del fármaco auristatina o maitansinoide.

40. El conjugado de anticuerpo con fármacos que comprende el anticuerpo diseñado con cisteína de la reivindicación 33 (Ab), y una fracción del fármaco auristatina o maitansinoide (D), en la que el anticuerpo diseñado con cisteína se une por medio de uno o más aminoácidos de cisteína libre a través de una porción de enlazador (L) a D, el compuesto que posee Fórmula I:

Ab–(L–D)p

donde p es 1, 2, 3, o 4.

41. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 40, donde p es 2.

42. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 40, donde L tiene la fórmula:

$$—A_a—W_w—Y_y—$$

donde:

A es una unidad de extensión unida covalentemente a un tiol de cisteína del anticuerpo diseñado con cisteína (Ab),

a es 0 ó 1,

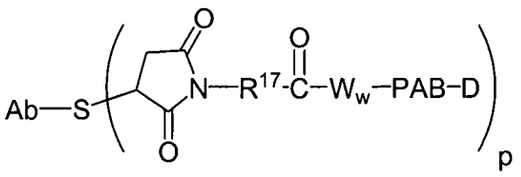
cada W es independientemente una unidad de aminoácidos,

w es un número entero que oscila entre 0 y 12,

Y es una unidad espaciadora unida covalentemente a una fracción de fármaco y

y es 0, 1 ó 2.

43. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 42 que tiene la fórmula:

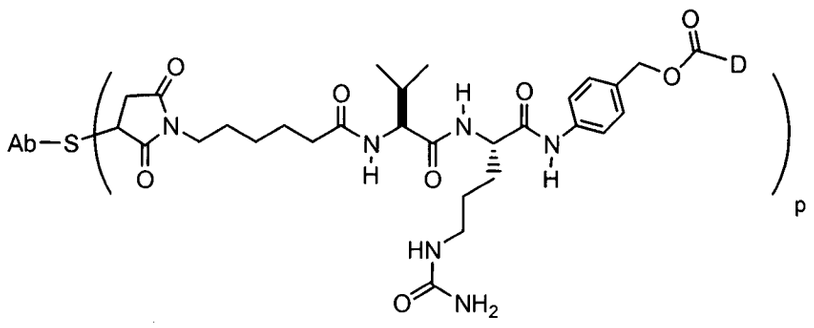


donde PAB es un para-aminobenzilcarbamoil y ^{17}R es un radical divalente seleccionado de $(CH_2)_r$, C_3-C_8 carbociclilo, O–$(CH_2)_r$, arileno, $(CH_2)_r$–arileno, –arileno–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbociclilo), (C_3-C_8 carbociclilo)–$(CH_2)_r$, C_3-C_8 heterociclilo, $(CH_2)_r$–(C_3-C_8 heterociclilo), –(C_3-C_8 heterociclilo)–$(CH_2)_r$–, –$(CH_2)_r$C(O)$NR^b(CH_2)_r$–, –$(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$–CH_2–, –$(CH_2)_r$C(O)$NR^b(CH_2CH_2O)_r$–, –$(CH_2)_r$C(O)$NR^b(CH_2CH_2O)_r$–CH_2–, –$(CH_2CH_2O)_r$C(O)$NR^b(CH_2CH_2O)_r$–, –$(CH_2CH_2O)_r$C(O)$NR^b(CH_2CH_2O)_r$–CH_2– y –$(CH_2CH_2O)_r$C(O)$NR^b(CH_2)_r$– ; donde R^b es H, C_1-C_6 alquilo, fenilo o bencilo y r es independientemente un número entero que oscila entre 1 y 10.

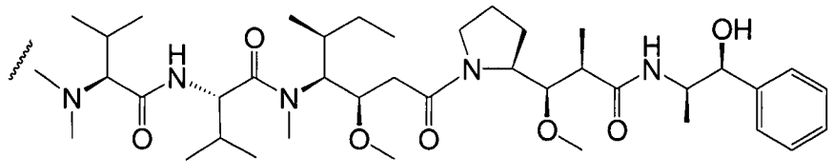
44. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 42, donde Ww es valina-citrulina.

45. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 42, donde ^{17}R es $(CH_2)_5$ o $(CH_2)_2$.

46. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 42 que tiene la fórmula:



47. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 40, donde D es MMAE, con la estructura:



donde la línea ondulada indica el sitio de unión al enlazador L.

48. Una formulación farmacéutica que comprende el conjugado de fármaco y anticuerpo de la reivindicación 39, y un diluyente, un vehículo o un excipiente farmacéuticamente aceptable.

49. Un método para el tratamiento del cáncer que comprende la administración a un paciente de la formulación farmacéutica de la reivindicación 48.

50. El método de la reivindicación 49, donde el cáncer es seleccionado a partir del grupo compuesto por el cáncer de próstata, cáncer de pulmón, cáncer de colon, cáncer de vejiga, cáncer de ovario y sarcoma de Ewing.

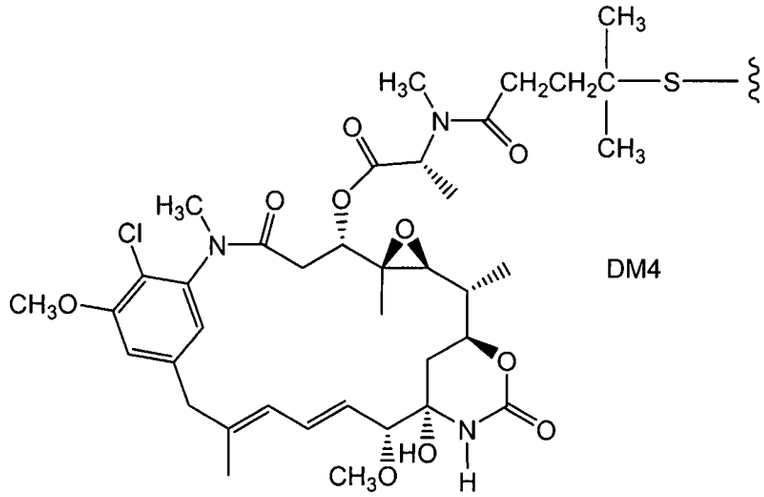
51. El método de la reivindicación 49, donde se administra al paciente un agente quimioterapéutico en combinación con el compuesto conjugado de fármaco y anticuerpo, donde el agente quimioterapéutico se selecciona entre letrozol, cisplatina, carboplatina, taxol, paclitaxel, oxaliplatina, doxetaxel, 5-FU, leucovorina, lapatinib y gemcitabina.

52. El anticuerpo diseñado con cisteína de la reivindicación 33, donde el anticuerpo contiene, una cisteína en una o más posiciones seleccionadas a partir de 15, 43, 110, 144, 168 y 205 de la cadena ligera de acuerdo con la convención de la numeración de Kabat y 41, 88, 115, 118, 120, 171, 172, 282, 375, y 400 de la cadena pesada según la convención de numeración de EU.

53. El anticuerpo de la reivindicación 52, en el que la cisteína está en la posición 205 de la cadena ligera.

54. El anticuerpo de la reivindicación 52, en el que la cisteína está en la posición 118 de la cadena pesada.

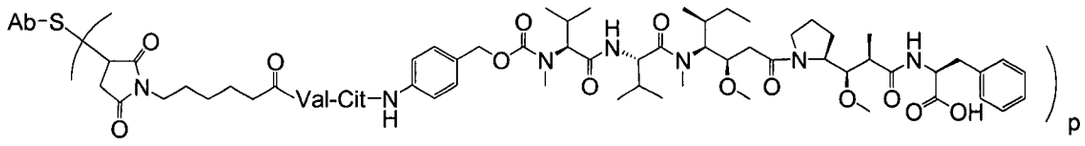
55. El compuesto del conjugado de fármaco y anticuerpo de la reivindicación 40 en el que D es DM4, teniendo la estructura:



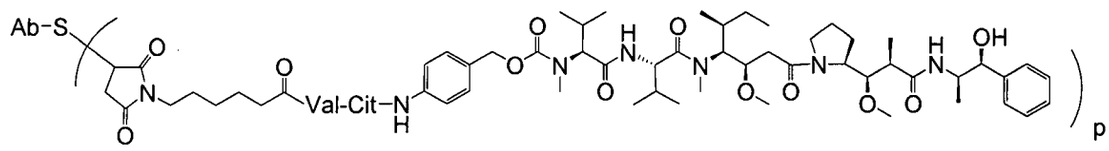
DM4

en el que la línea ondulada indica el sitio de unión al ligando L.

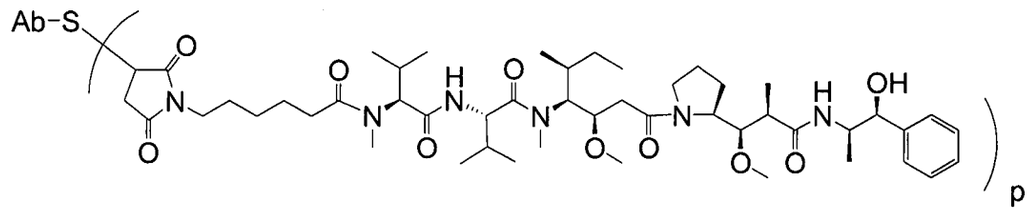
56. El compuesto de conjugado de anticuerpo con fármacos seleccionado de las estructuras:



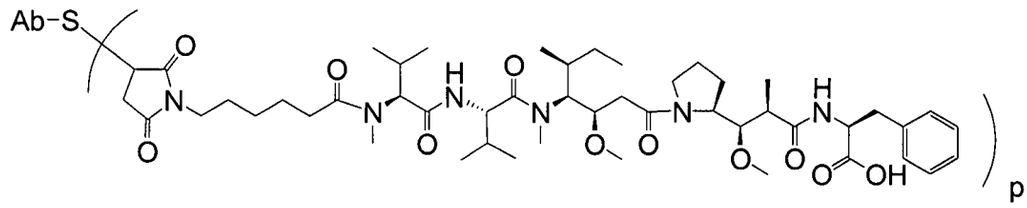
Ab-MC-vc-PAB-MMAF



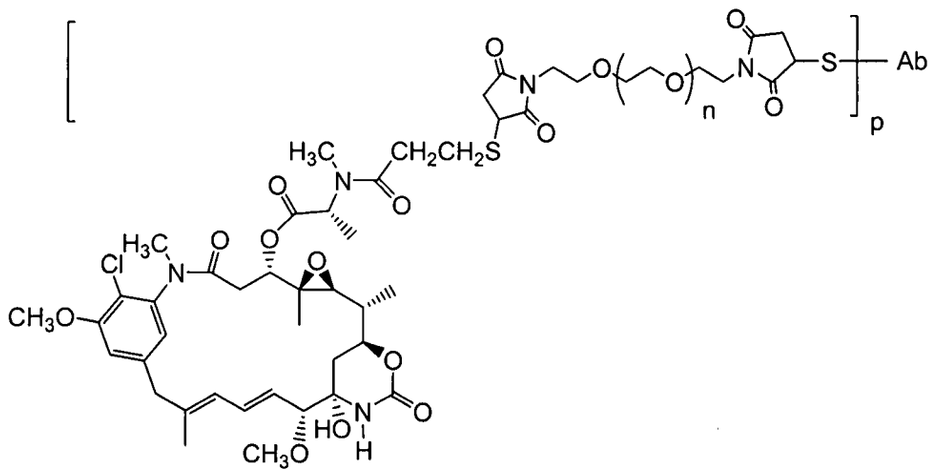
Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF



Ab-BMPEO-DM1

donde Val es valina, Cit es citrulina, p es 1, 2, 3 ó 4 y Ab es anticuerpo anti-STEAP-1 de la reivindicación 33.

57. El anticuerpo de la reivindicación 3, donde la CL comprende:

(1) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:90;

(2) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:92;

(3) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:93;

(4) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:94;

(5) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:95;

(6) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:96;

(7) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:97;

(8) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:98;

(9) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:99;

(10) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:100; y

(11) una región de cadena ligera que comprende la secuencia de aminoácido de SEC ID NO:101.

Schematic Diagram of STEAP-1 in the Cell Membrane

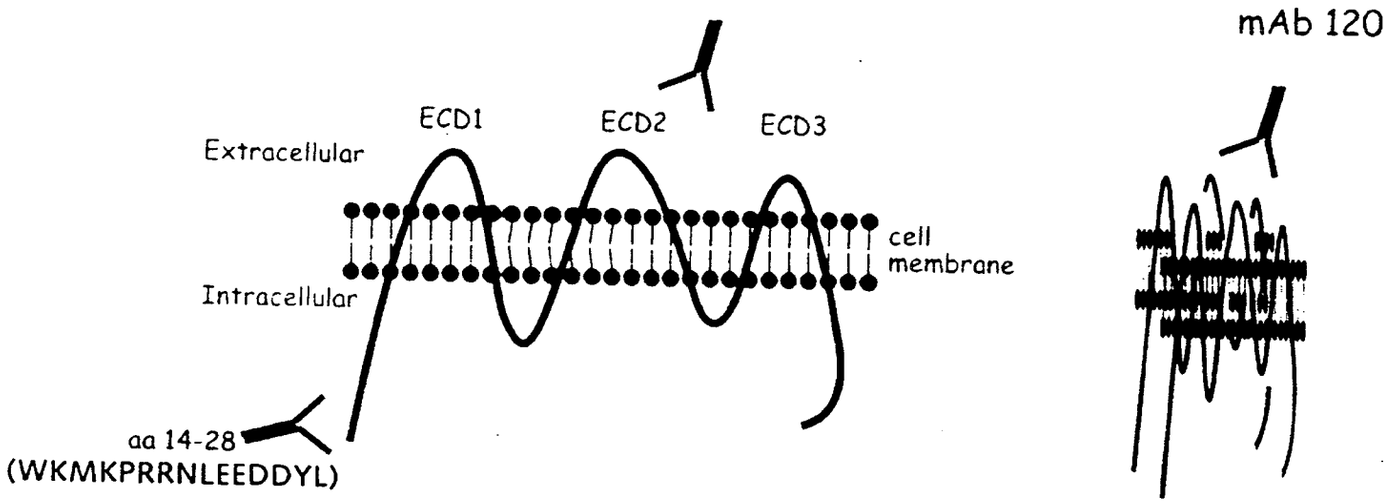


Figure 12

Industria y Comercio
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
EXTRACTO PARA PUBLICACIÓN
SOLICITUD DE PATENTE DE INVENCIÓN PCT

(21) N° de solicitud: **09-034.904**

(22) Fecha de solicitud: 03-04-09

(30) Prioridad

(31) *No. Prioridad*	(32) *Fecha*	(33) *País*
60/863,295	27-10-06	US
60/868,707	05-12-06	US
60/921,300	30-03-07	US
60/937,857	29-06-07	US

(51) Int Cl: C07K 16/30

(71)Solicitante(s) **GENENTECH, INC.**

(72) Inventor(es) DENNOS, Mark, S.; RUBINFELD, Bonnee; POLAKIS, Paul y JAKOBOVITS, Aya.

(74) Apoderado: **ALICIA LLOREDA RICAURTE**

(85) Fecha limite inicio fase nacional: 27-05-09

(86) Datos relativos a la presentación de la solicitud PCT
Fecha de presentación de la solicitud: **26/10/07**
No. de solicitud ***PCT/US2007/082726***

(87) Publicación internacional
Fecha: **02/05/08**
N° publicación: ***WO 2008/052187 A3***

(54) Titulo: **"ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS"**

Resumen - Reivindicación(es):

Reivindicación número 1:

Un anticuerpo humanizado monoclonal que se une a STEAP-1, donde el anticuerpo comprende una cadena pesada (HC) que comprende:

(1) una HVR-H1 que contiene la secuencia de aminoácidos de SEC ID NO:14;

(2) una HVR-H2 que contiene la secuencia de aminoácidos de SEC ID NO:15;

(3) una HVR-H3 que contiene la secuencia de aminoácidos de SEC ID NO:16; y

(4) una HC-FR1 que contiene la secuencia de aminoácidos de SEC ID NO:25.

Industria y Comercio
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
TARJETA ARCHIVO TEMATICO

PATENTE DE INVENCION ____ PCT _X_ MODELO DE UTILIDAD ____ DISEÑO INDUSTRIAL ___

(21) N° de solicitud: **09-034.904** (22) Fecha de solicitud: 03-04-09

(51) Clasificación Internacional : C07K 16/30

(71) Solicitante(s) **GENENTECH, INC.**

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60/921,300	**30-03-07**	**US**
60/937,857	**29-06-07**	**US**

(85) Fecha límite inicio fase nacional: 27-05-09

(86) Datos relativos a la presentación de la solic. PCT
Fecha de presentación de la solicitud: 26-10-07
No. de solicitud ***PCT/US2007/082726***

(87) Publicación internacional:
Fecha: 02-05-08
No. Publicación : **WO 2008/052187 A3**

FIGURA CARACTERISTICA
6 x 6 cm

(54) Titulo: **"ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS"**

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
LLOREDA RICAURTE ALICIA
Apoderado(a) y/o Representante de
GENENTECH INC

REFERENCIA	Radicación No.	09 34904
	Solicitud internacional	PCT/US2007/082726
	Fecha inicio fase nacional	27/05/2009
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	08474

Teniendo en cuenta que el capítulo reivindicatorio presentado excede de quince reivindicaciones, es necesario que cancele la tasa establecida por cada reivindicación adicional presentada, de conformidad con lo dispuesto en la resolución de tasas vigente para la fecha de presentación de la solicitud, so pena de que no sean tenidas en cuenta dentro del presente trámite administrativo.

En consecuencia, con fundamento en lo previsto por el artículo 12 del Código Contencioso Administrativo, se le requiere para que dentro del término de dos (2) meses siguientes a la fecha de notificación del presente oficio atienda el requerimiento aquí señalado. Lo anterior, con el alcance que para tal efecto establece el artículo 13 de la misma codificación, respecto de las reivindicaciones adicionales.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del titulo primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 24 JUL. 2009
jfernand

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