Organizacion y Digitalizacion CSA Ltda

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

SIC

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621

DELEGATURA PROPIEDAD INDUSTRIAL

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCION

ENTRADA FASE NACIONAL

08-127238

21. **EXPEDIENTE No.** _____

54. **TITULO** ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

51. **CLASIFICACION INTERNACIONAL** _____

71. **SOLICITANTE** GENENTECH, INC.

DOMICILIO SAN FRANCISCO, CA, ESTADOS UNIDOS DE AMERICA

74. **APODERADO** JUAN PABLO CADENA SARMIENTO

22. **SANTAFE DE BOGOTA, D.C.** _____

PETITORIO

AS: 108.985

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 08-127238- -00000-0000
Fecha: 2008-11-28 17:05:43 Dep: 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 799

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

30 Dic 2008

1 SOLICITUD DE:

☒ Patente de Invención. ☐ Patente de Modelo de Utilidad.

☒ Capítulo I. ☐ Capítulo II.

2 SOLICITANTE (71)	Nombre: **GENENTECH, INC.** Dirección: 1 Dna Way, South San Francisco, California 94080, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos de América Lugar de Constitución: Estados Unidos de América Teléfono: Fax: E-mail	**IDENTIFICACIÓN** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual_____ Número _____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Cra. 7 No. 71-21 Torre B – Piso 4, Bogotá, Colombia. Teléfono: 744-2200 Fax: 742-2200 E-mail:	**IDENTIFICACIÓN** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número 80.410.337 TP 57492
4 INVENTOR (ES) (72)	**Nombre:** Allen J. EBENS Jr., Alane M. GRAY, Wei-Ching LIANG, Yan WU, Shang-Fan YU. **Dirección**: 1932 Arroyo Avenue, San Carlos, California 94070, US; 122 Kestrel Court, Brisbane, California 94005, US; 840 Sea Spray Lane, #112, Foster City, California 94404, US; 1160 Blythe Street, Foster City, California 94404, US; 1103 Sandalwood Lane, Milpitas, California 95035 US (Respectivamente). **Nacionalidad o Domicilio:** Estados Unidos de América (Respectivamente).	

5 PCT (86)

Solicitud Internacional No. PCT/US2007/069889 Fecha 29-05-2007
Publicación Internacional No WO 2007/140371 A3 Fecha 06-12-2007

6 Título (54): ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

7 Clasificación Internacional (51): _____

8 Prioridad SI ☒ NO ☐

(33) País de Origen	(32) Fecha	(31) Número de Solicitud
US	30-05-2006	60/809,328
US	29-03-2007	60/908,941
US	13-04-2007	60/911,829

9 Comprobante de pago No. 08 – 81; 879 **Fecha** 27-11-2008

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

10 **ANEXOS**

- [x] Comprobante de pago de la tasa de presentación de la solicitud. $ 501.000
- [x] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [x] Poderes, si fuere el caso. **Fotocopia autenticada**
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [ ] Declaraciones.
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar efectuado por la IPEA.
- [ ] Traducción del examen preliminar efectuado por la IPEA.
- [ ] De ser el caso, copia del contrato de acceso.
- [x] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- [ ] De ser el caso, certificado de depósito del material biológico.
- [ ] Arte final 12x12.
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- [x] Otros: Tarjeta de Propietarios
  Documentos PCT

11 FIGURA CARACTERÍSTICA:

12 Solicito la concesión de la patente.

NOMBRE: JUAN PABLO CADENA SARMIENTO

FIRMA:

C.C. 80.410.337 de Usaquen T.P. 57492 del C.S.J.

Instrucciones para la presentación de los anexos, ver reverso de esta página.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

PATENTE [X] PCT/US2007/069889 MODELO DE UTILIDAD [ ] DISEÑO INDUSTRIAL [ ]

SOLICITANTE/S. RAZON SOCIAL O APELLIDOS Y NOMBRES GENENTECH, INC.

DOMICILIO SAN FRANCISCO, CA, ESTADOS UNIDOS DE AMERICA

APODERADO JUAN PABLO CADENA SARMIENTO

TITULO ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

CLASIFICACION

EXPEDIENTE No. FECHA DE SOLICITUD ROLLO MICROFILM

CERTIFICADO No. FECHA DE CONCESION VIGENCIA DESDE HASTA

PRORROGA CAMBIO DE NOMBRE

TRASPASO A

INVENTOR (ES) ALLEN J. EBENS JR., ALANE M. GRAY, WEI-CHING LIANG, YAN WU,

OTROS SHANG-FAN YU.

OFICINA DE COMUNICACIONES

108 935

4

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 08-127238- -00000-0000
Fecha: 2008-11-28 17:05:43 Dep: 2020 NUECREACIONI
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 81,879
FECHA : OCTUBRE 15 DE 2008

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
BRIGARD & CASTRO LTDA	CONSIGNACION	BANCO DE BOGOTA	062754387	3247646	15/10/2008	34,818,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	501,000.00
		TOTAL : $	501,000.00

SON: QUINIENTOS UN MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

108985

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

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2007 (06.12.2007)

PCT

(10) International Publication Number
WO 2007/140371 A3

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 47/48* (2006.01)

(21) International Application Number:
PCT/US2007/069889

(22) International Filing Date: 29 May 2007 (29.05.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/809,328	30 May 2006 (30.05.2006)	US
60/908,941	29 March 2007 (29.03.2007)	US
60/911,829	13 April 2007 (13.04.2007)	US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EBENS, Allen, J., Jr.** [US/US]; 1932 Arroyo Avenue, San Carlos, California 94070 (US). **GRAY, Alane M.** [US/US]; 122 Kestrel Court, Brisbane, California 94005 (US). **LIANG, Wei-Ching** [CN/US]; 840 Sea Spray Lane, #112, Foster City, California 94404 (US). **WU, Yan** [US/US]; 1160 Blythe Street, Foster City, California 94404 (US). **YU, Shang-Fan** [CN/US]; 1103 Sandalwood Lane, Milpitas, California 95035 (US).

(74) Agents: **CONLEY, Deirdre L.** et al.; c/o GENENTECH, INC., 1 DNA Way MS 49, South San Francisco, California 94080 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(88) Date of publication of the international search report:
14 February 2008

(48) Date of publication of this corrected version:
2 October 2008

(15) Information about Correction:
see Notice of 2 October 2008

(54) Title: ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

(57) Abstract: Anti-CD22 antibodies and immunoconjugates thereof are provided. Methods of using anti-CD22 antibodies and immunoconjugates thereof are provided.

WO 2007/140371 A3

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2007 (06.12.2007)

PCT

(10) International Publication Number
WO 2007/140371 A3

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 47/48* (2006.01)

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(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **EBENS, Allen, J., Jr.** [US/US]; 1932 Arroyo Avenue, San Carlos, California 94070 (US). **GRAY, Alane M.** [US/US]; 122 Kestrel Court, Brisbane, California 94005 (US). **LIANG, Wei-Ching** [CN/US]; 840 Sea Spray Lane, #112, Foster City, California 94404 (US). **WU, Yan** [US/US]; 846 Andromeda Lane, Foster City, California 94404 (US). **YU, Shang-Fan** [CN/US]; 1103 Sandalwood Lane, Milpitas, California 95035 (US).

(74) Agents: **CONLEY, Deirdre L.** et al.; c/o GENENTECH, INC., 1 DNA Way MS 49, South San Francisco, California 94080 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
14 February 2008

WO 2007/140371 A3

(54) Title: ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

(57) Abstract: Anti-CD22 antibodies and immunoconjugates thereof are provided. Methods of using anti-CD22 antibodies and immunoconjugates thereof are provided.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/069889

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/042240 A (WYETH CORP [US]; IYER ANAND [US]; DUNUSSI-JOANNOPOULOS KYRIAKI [US]) 20 April 2006 (2006-04-20) Seq. 28 is 92.24% identical to Seq. 87 of application	1-238
X	WO 03/072736 A (UNIV DUKE [US]; TEDDER THOMAS F [US]) 4 September 2003 (2003-09-04) Seq. 29 is anti-CD22 light chain and comprises Seq. 12 of application the whole document	1-238

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2007	12/12/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sommerfeld, Teresa

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/069889

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/093320 A (CELLTECH R & D LTD. [GB]; POPPLEWELL ANDREW GEORGE [GB]; TICKLE SIMON P) 13 November 2003 (2003-11-13) Seq. 28 is 92.24% identical to Seq. 87 of application the whole document	1-238
A	WO 2005/113003 A (GENENTECH INC [US]; CHAN ANDREW C [US]; GONG QIAN [US]; MARTIN FLAVIUS) 1 December 2005 (2005-12-01) Seq.32 is 95.35% identical to Seq. 88 of application	
A	LEONARD JOHN P ET AL: "Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 AUG 2004, vol. 10, no. 16, 15 August 2004 (2004-08-15), pages 5327-5334, XP002459865 ISSN: 1078-0432	
A	DIJOSEPH J F ET AL: "ANTIBODY-TARGETED CHEMOTHERAPY WITH CMC-544: A CD22-TARGETED IMMUNOCONJUGATE OF CALICHEAMICIN FOR THE TREATMENT OF B-LYMPHOID MALIGNANCIES" BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, US, vol. 103, no. 5, March 2004 (2004-03), pages 1807-1814, XP008060153 ISSN: 0006-4971	
A	WO 2005/117986 A (GENENTECH INC [US]; EBENS JR ALLEN J [US]; JACOBSON FREDERIC S [US]; P) 15 December 2005 (2005-12-15)	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/069889

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 142, 143, 145, 146, 148-152, 154, 221, 223-225 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/069889

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006042240 A	20-04-2006	AR 052774 A1	04-04-2007
		AU 2005295041 A1	20-04-2006
		CA 2582919 A1	20-04-2006
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		US 2003235869 A1	25-12-2003
		US 2007059307 A1	15-03-2007
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		BR PI0508762 A	14-08-2007
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		EP 1735000 A2	27-12-2006
		KR 20070012408 A	25-01-2007
WO 2005117986 A	15-12-2005	AU 2005249490 A1	15-12-2005
		CA 2567520 A1	15-12-2005
		EP 1753463 A2	21-02-2007
		KR 20070037575 A	05-04-2007

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(19) World Intellectual Property Organization
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(51) International Patent Classification:
C07K 16/28 (2006.01)

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

(57) Abstract: Anti-CD22 antibodies and immunoconjugates thereof are provided. Methods of using anti-CD22 antibodies and immunoconjugates thereof are provided.

WO 2007/140371 A2

Patent Docket No.: P2262R1

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

This is a non-provisional application filed under 37 CFR Section 1.53(b) claiming priority under 35 USC Section 119(e) to U.S. Provisional Applications Serial Nos. 60/809,328, filed 30 May 2006, 60/908,941, filed 29 March 2007, and 60/911,829, filed 13 April 2007, the entire contents of which applications are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to anti-CD22 antibodies and immunconjugates thereof. The invention further relates to methods of using anti-CD22 antibodies and immunconjugates thereof.

BACKGROUND

Lymphocytes are one of many types of white blood cells produced in the bone marrow during the process of hematopoiesis. There are two major populations of lymphocytes: B lymphocytes (B cells) and T lymphocytes (T cells). The lymphocytes of particular interest herein are B cells.

B cells mature within the bone marrow and leave the marrow expressing an antigen-binding antibody on their cell surface. When a naive B cell first encounters the antigen for which its membrane-bound antibody is specific, the cell begins to divide rapidly and its progeny differentiate into memory B cells and effector cells called "plasma cells." Memory B cells have a longer life span and continue to express membrane-bound antibody with the same specificity as the original parent cell. Plasma cells do not produce membrane-bound antibody but instead produce the antibody in a form that can be secreted. Secreted antibodies are the major effector molecule of humoral immunity.

B cell-related disorders include, but are not limited to, malignant lymphoma (Non-Hodgkin's Lymphoma, NHL), multiple myeloma, and chronic lymphocytic leukemia (CLL, B cell leukemia (CD5+ B lymphocytes). Non-Hodgkin's lymphomas (NHLs), a heterogeneous group of cancers principally arising from B lymphocytes, represent approximately 4% of all newly diagnosed cancers (Jemal, A. et al., CA-Cancer J Clin, 52:

23-47, (2002)). Aggressive NHL comprises approximately 30–40% of adult NHL (Harris, N.L. et al., Hematol. J. 1:53-66 (2001)) and includes diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), peripheral T-cell lymphoma, and anaplastic large cell lymphoma. Frontline combination chemotherapy cures less than half of the patients with aggressive NHL, and most patients eventually succumb to their disease (Fisher, R.I. Semin. Oncol. 27(suppl 12): 2-8 (2000)).

B cell-related disorders also include autoimmune diseases. Autoimmune diseases remain clinically important diseases in humans. As the name implies, autoimmune diseases act through the body's own immune system. While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) to the body's endogenous proteins. Physicians and scientists have identified more than 70 clinically distinct autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, vasculitis, immune-mediated diabetes, and lupus such as systemic lupus erythematosus. While many autoimmune diseases are rare – affecting fewer than 200,000 individuals – collectively, these diseases afflict millions of Americans, an estimated five percent of the population, with women disproportionately affected by most diseases. The chronic nature of these diseases leads to an immense social and financial burden.

Cytotoxic agents which target B cell surface antigens are an important focus of B cell-related cancer therapies. One such B cell surface antigen is CD20. Rituximab (Rituxan; Genentech, Inc. (South San Francisco, CA) and IDEC Pharmaceutical Corp. (San Diego, CA)), a chimeric (mouse/human) anti-CD20 monoclonal antibody, was the first therapeutic antibody approved by the United States Food and Drug Administration for treatment of relapsed or refractory low-grade or follicular NHL (Leonard, J.P. et al., Clin. Canc. Res. 10:5327-5334 (2004)).

Other B-cell antigens, such as CD19, CD22, and CD52, represent targets of therapeutic potential for treatment of lymphoma (Grillo-Lopez A.J. et al., Curr Pharm Biotechnol, 2:301-11, (2001)) . CD22 is a 135-kDa B-cell-restricted sialoglycoprotein expressed on the B-cell surface only at the mature stages of differentiation (Dorken, B. et al., J. Immunol. 136:4470-4479 (1986)). The predominant form of CD22 in humans is CD22beta which contains seven immunoglobulin superfamily domains in the extracellular

domain (Figure 1) (Wilson, G.L. et al., J. Exp. Med. 173:137-146 (1991)). A variant form, CD22 alpha, lacks immunoglobulin superfamily domains 3 and 4 (Stamenkovic, I. and Seed, B., Nature 345:74-77 (1990)). Ligand-binding to human CD22 has been shown to be associated with immunoglobulin superfamily domains 1 and 2 (also referred to as epitopes 1 and 2) (Engel, P. et al., J. Exp. Med. 181:1581-1586, 1995).

In B-cell NHL, CD22 expression ranges from 91% to 99% in the aggressive and indolent populations, respectively (Cesano, A. et al., Blood 100:350a (2002)). CD22 may function both as a component of the B-cell activation complex (Sato, S. et al., Semin. Immunol. 10:287-296 (1998)) and as an adhesion molecule (Engel, P l t al., J. Immunol. 150:4719-4732 (1993)). The B cells of CD22-deficient mice have a shorter life span and enhanced apoptosis, which suggests a key role of this antigen in B-cell survival (Otipoby, K.L. et al., Nature (Lond) 384:634-637 (1996)). After binding with its natural ligand(s) or antibodies, CD22 is rapidly internalized, providing a potent costimulatory signal in primary B cells and proapoptotic signals in neoplastic B cells (Sato, S. et al., Immunity 5:551-562 (1996)).

Anti-CD22 antibodies have been studied as potential therapies for B cell cancers and other B cell proliferative diseases. Such anti-CD22 antibodies include RFB4 Mansfield, E. et al., Blood 90:2020-2026 (1997)), CMC-544 (DiJoseph, J.F., Blood 103:1807-1814 (2004)) and LL2 (Pawlak-Byczkowska, E.J. et al., Cancer Res. 49:4568-4577 (1989)). The LL2 antibody (formerly called HPB-2) is an IgG2a mouse monoclonal antibody directed against the CD22 antigen (Pawlak-Byczkowska, E.J. et al. (1989), supra). In vitro immunohistological evaluations demonstrated reactivity of the LL2 antibody with 50 of 51 B-cell NHL specimens tested, but not with other malignancies or normal nonlymphoid tissues (Pawlak-Byczkowska (1989), supra; Stein, R. et al., Cancer Immunol. Immunother. 37:293-298 (1993)).

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). 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 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).

Anti-CD22 antibody-toxin conjugates have also been studied as potential therapeutic compounds. For example, early reports described ricin A chain-containing immunotoxins directed against anti-CD22 as potential anti-cancer agents (May, R.D. et al., Chemical Abstracts 106(21):168656x pages 35-36 (1987); Ghetie, M.A. et al., Cancer Research 48:2610-2617 (1988); and Amlot, P.L. et al., Blood 82(9):2624-2633 (1993)). Where the toxin was a radioisotope, Epratuzumab, the humanized (CDR-grafted) IgG1 version of LL2, has shown evidence of therapeutic activity for the radioimmunoconjugate (Juweid, M.E. et al., Clin. Cancer Res. 5 (Suppl 10):3292s-3303s (1999); Griffiths, G.L. et al., J. Nucl. Med. 44:77-84 (2003); Linden, O. et al., Clin. Cancer Res. 5(suppl 10):3287s-3291s (1999)).

There exists a need in the art for additional drugs to treat various B cell-related cancers such as lymphomas such as non-Hodgkin's lymphoma and other B cell proliferative disorders. Particularly useful drugs for this purpose include B cell-targeted anti-CD22 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-CD22 antibodies and methods of using the same.

In one aspect, an antibody that binds to CD22 is provided, wherein the antibody comprises at least one, two, three, four, five, or six HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;

(4) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:10;

(5) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In another aspect, an antibody that binds to CD22 comprises (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:10, 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:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In another aspect, an antibody that binds to CD22 comprises (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:9, 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:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In another aspect, an antibody that binds to CD22 comprises (a) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6, 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:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:9;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(5) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In another aspect, an antibody that binds to CD22 comprises (a) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6, 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:2;

(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;

(3) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:10;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(5) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In one embodiment, the antibody comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO:10. In one embodiment, the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2 and an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4. In one embodiment, the antibody

further comprises an HVR-L2 comprising the amino acid sequence of SEQ NO:12 and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:14.

In certain embodiments, any of the above antibodies further comprises at least one framework selected from a VH subgroup III consensus framework and a VL subgroup I consensus framework.

In one aspect, an antibody that binds to CD22 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 NO:16. In one embodiment, the antibody comprises a heavy chain variable domain of SEQ ID NO:16.

In one aspect, 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:17. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:17.

In one aspect, 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:18. In one embodiment, the antibody comprises a light chain variable domain of SEQ ID NO:18.

In one embodiment, 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%, at least 99%, or 100% sequence identity to an amino acid sequence of SEQ ID NO:16 and 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%, at least 99%, or 100% sequence identity to an amino acid sequence of SEQ ID NO:17. In one embodiment, 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%, at least 99%, or 100% sequence identity to an amino acid sequence of SEQ ID NO:16 and 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%, at least 99%, or 100% sequence identity to an amino acid sequence of SEQ ID NO:18. In one embodiment, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:16, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:17. In one embodiment, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:16, and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:18.

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-CD22 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 CD22 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 CD22 comprising domain 1 or domain 2 or domains 1 and 2. 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 B cell. In one embodiment the cancer cell is a B cell.

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 CD22 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 CD22, and detecting whether a complex is formed between the antibody and CD22. In one embodiment, the biological sample comprises B cells. In one embodiment, the biological sample is from a mammal experiencing or suspected of experiencing a B cell disorder and/or a B cell

proliferative disorder including, but not limited to, 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.

In one aspect, a method of diagnosing a cell proliferative disorder associated with increased expression of CD22 is provided, the method comprising contacting a test cell with any of the above antibodies; determining the level of expression of CD22 by detecting binding of the antibody to CD22; and comparing the level of expression of CD22 by the test cell with the level of expression of CD22 by a control cell, wherein a higher level of expression of CD22 by the test cell as compared to the control cell indicates the presence of a cell proliferative disorder associated with increased expression of CD22. In one embodiment, the test cell is a cell from a patient suspected of having a cell proliferative disorder, such as a B-cell proliferative disorder. In one embodiment, the cell proliferative disorder is selected from B cell disorders including but not limited to 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. In one embodiment, the method comprises determining the level of expression of CD22 on the surface of the test cell and comparing the level of expression of CD22 on the surface of the test cell with the level of expression of CD22 on the surface of the control cell.

In one aspect, a method of diagnosing a cell proliferative disorder associated with an increase in cells, such as B cells, expressing CD22 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 CD22; 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 CD22-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 CD22.

In one aspect, a method of detecting soluble CD22 in blood or serum, the method comprising contacting a test sample of blood or serum from a mammal suspected of experiencing a B cell proliferative disorder with an anti-CD22 antibody of the invention and detecting a increase in soluble CD22 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 B cell proliferative disorder associated with an increase in soluble CD22 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). Any form of anti-CD22 antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab, referred to herein as "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." It should be noted that a single site mutation yields a single engineered cysteine residue in a ThioFab, while a single site mutation yields two engineered cysteine residues in a ThioMab, due to the dimeric nature of the IgG antibody. The cysteine engineered anti-CD22 antibodies of the invention include monoclonal antibodies, humanized or chimeric monoclonal antibodies, and antigen-binding fragments of antibodies, fusion polypeptides and analogs that preferentially bind cell-associated CD22 polypeptides. A cysteine engineered antibody may alternatively comprise an antibody comprising a cysteine at a position disclosed herein in the antibody or Fab, resulting from the sequence design and/or selection of the antibody, without necessarily altering a parent antibody, such as by phage display antibody design and selection or through de novo design of light chain and/or heavy chain framework sequences and constant regions. A cysteine engineered antibody comprises one or more free cysteine amino acids having a thiol reactivity value in the ranges of 0.6 to 1.0; 0.7 to 1.0 or 0.8 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).

In an embodiment, a cysteine engineered anti-CD22 antibody of the invention comprises an engineered cysteine at any one of the following positions, where the position is number according to Kabat et al. in the light chain (see Kabat et al (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) and according to EU numbering in the heavy chain (including the Fc region) (see see Kabat et al. (1991), supra) , wherein the light chain constant region depicted by underlining in Figure 17A begins at position 108 (Kabat numbering) and the heavy chain constant region depicted by underling in Figures 17B and 17C begins at position 118 (EU numbering). The position may also be referred to by its position in sequential numbering of the amino acids of the full length light chain or heavy chain shown in Figures 17A-17C. According to one embodiment of the invention, an anti-CD22 antibody comprises an engineered cysteine at LC-V205C (Kabat number: Val 205; sequential number 210 in Figure 17A engineered to be Cys at that position). The engineered cysteine in the light chain is shown in bold, double underlined text in Figure 17A. According to one embodiment, an anti-CD22 antibody comprises an engineered cysteine at HC-A118C (EU number: Ala 118; sequential number 121 in Figure 17B engineered to be Cys at that position). The engineered cysteine in the heavy chain is shown in bold, double underlined text in Figure 17B. According to one embodiment, an anti-CD22 antibody comprises an engineered cysteine at Fc-S400C (EU number: Ser 400; sequential number 403 in Figure 17C engineered to be Cys at that position). The engineered cysteine in the Fc region of the heavy chain is shown in bold, double underlined text in Figure 17C. In other embodiments, the engineered cysteine of the heavy chain (including the Fc region) is at any one of the following positions (according to EU numbering): 41, 88, 116, 118, 120, 171, 282, 375, or 400. Thus, changes in the amino acid at these positions for a parent anti-CD22 antibody of the invention are: A41C, A88C, S116C, A118C, T120C, A171C, V282C, S375C, or S400C. In other embodiments, the engineered cysteine of the light chain is at any one of the following positions (according to Kabat numbering): 15, 43, 110, 144, 168, 205. Thus, changes in the amino acid at these postitions for a parent anti-CD22 antibody of the invention are: V15C, A43C, V110C, A144C, S168C, or V205C.

A cysteine engineered anti-CD22 antibody comprises one or more free cysteine amino acids wherein the cysteine engineered anti-CD22 antibody binds to a CD22 polypeptide and is prepared by a process comprising replacing one or more amino acid residues of a parent anti-CD22 antibody by cysteine wherein the parent antibody comprises at least one HVR sequence selected from:

(a) an HVR-L1 sequence RSSQSIVHSNGNTFLE (SEQ ID NO:9) or sequence RSSQSIVHSVGNTFLE (SEQ ID NO:10) (Figure 2B);

(b) an HVR-L2 sequence KVSNRFS SEQ ID NO:12 (Figure 2B);

(c) an HVR-L3 sequence FQGSQFPYT (SEQ ID NO:14) (Figure 2B);

(d) an HVR-H1 sequence GYEFSRSWMN (SEQ ID NO:2) (Figure 2A);

(e) an HVR-H2 sequence GRIYPGDGDTNYSGKFKG (SEQ ID NO:4 (Figure 2A); and

(f) an HVR-H3 sequence DGSSWDWYFDV (SEQ ID NO:6) (Figure 2A).

In a certain aspect, the invention concerns a cysteine engineered anti-CD22 antibody, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity, to a cysteine engineered antibody having a full-length amino acid sequence as disclosed herein, or a cysteine engineered antibody amino acid sequence lacking the signal peptide as disclosed herein.

In a yet further aspect, the invention concerns an isolated cysteine engineered anti-CD22 antibody comprising an amino acid sequence that is encoded by a nucleotide sequence that hybridizes to the complement of a DNA molecule encoding (a) a cysteine engineered antibody having a full-length amino acid sequence as disclosed herein, (b) a cysteine engineered antibody amino acid sequence lacking the signal peptide as disclosed herein, (c) an extracellular domain of a transmembrane cysteine engineered antibody protein, with or without the signal peptide, as disclosed herein, (d) an amino acid sequence encoded by any of the nucleic acid sequences disclosed herein or (e) any other specifically

defined fragment of a full-length cysteine engineered antibody amino acid sequence as disclosed herein.

In a specific aspect, the invention provides an isolated cysteine engineered anti-CD22 antibody without the N-terminal signal sequence and/or without the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as described in. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the cysteine engineered antibody and recovering the cysteine engineered antibody from the cell culture.

Another aspect of the invention provides an isolated cysteine engineered anti-CD22 antibody which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the cysteine engineered antibody and recovering the cysteine engineered antibody from the cell culture.

In other embodiments, the invention provides isolated anti-CD22 chimeric cysteine engineered antibodies comprising any of the herein described cysteine engineered antibody fused to a heterologous (non-CD22) polypeptide. Example of such chimeric molecules comprise any of the herein described cysteine engineered antibodies fused to a heterologous polypeptide such as, for example, an epitope tag sequence or a Fc region of an immunoglobulin.

The cysteine engineered anti-CD22 antibody may be a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, single-chain antibody or antibody that competitively inhibits the binding of an anti-CD22 polypeptide antibody to its respective antigenic epitope. Antibodies of the present invention may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin, including, for example, an auristatin, an antibiotic, a radioactive isotope, a nucleolytic enzyme, or the like. The antibodies of the present invention may optionally be produced in CHO cells or

bacterial cells and preferably inhibit the growth or proliferation of or induce the death of a cell to which they bind. For diagnostic purposes, the antibodies of the present invention may be detectably labeled, attached to a solid support, or the like.

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described anti-CD22 antibodies and anti-CD22 cysteine engineered antibodies. Host cells comprising any such vector are also provided. By way of example, the host cells may be CHO cells, E. coli cells, or yeast cells. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

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, 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. The tumor-associated antigen may be a cluster differentiation factor (i.e., a CD protein, including but not limited to CD22). Cysteine engineered anti-CD22 antibodies of the invention retain the antigen binding apability of their parent anti-CD22 antibody compounterparts. Thus, cysteine engineered anti-CD22 antibodies of the invention are capable of binding, preferably specifically, to CD22 antigens including human anti-CD22 isoforms beta and/or alpha, including when such antigens are expressed on the surface of cells, including, without limitation, B cells.

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 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; 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 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 4:193-204). Uses of labelled antibodies also include cell surface receptor binding assays, immmunocapture 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 (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.

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 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:42-46:

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	SEQ ID NO:42
QRLMEDICLPRWGCLWEDDF	SEQ ID NO:43

QRLIEDICLPRWGCLWEDDF SEQ ID NO:44

RLIEDICLPRWGCLWEDD SEQ ID NO:45

DICLPRWGCLW SEQ ID NO:46

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-CD22 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 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, Alcurites 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 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.

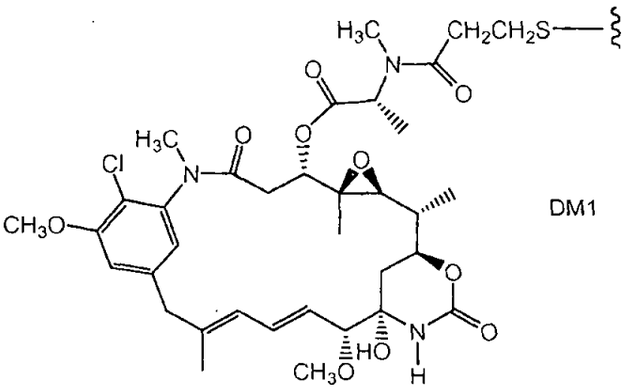
Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation 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.

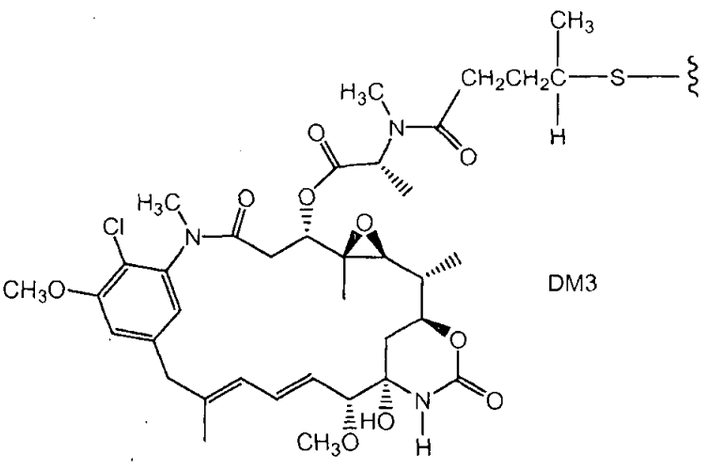
Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (US 4256746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (prepared by demethylation using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

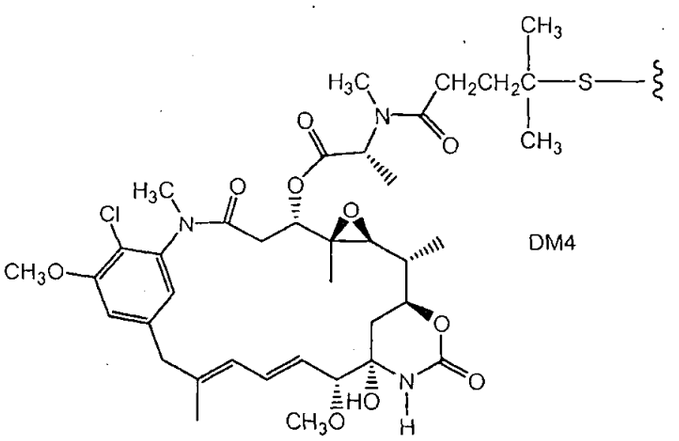
Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, having the structures:



DM1



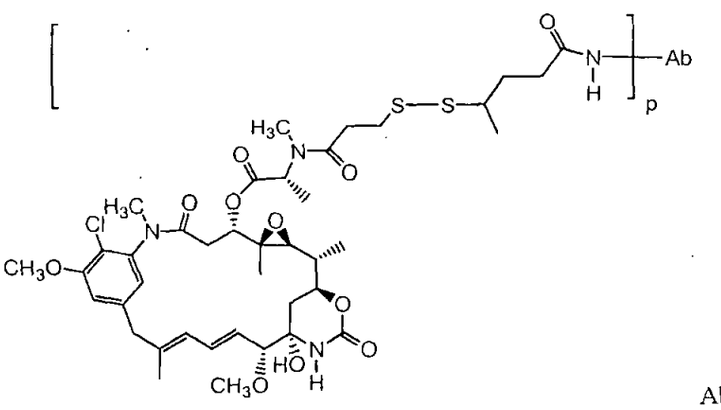
DM3



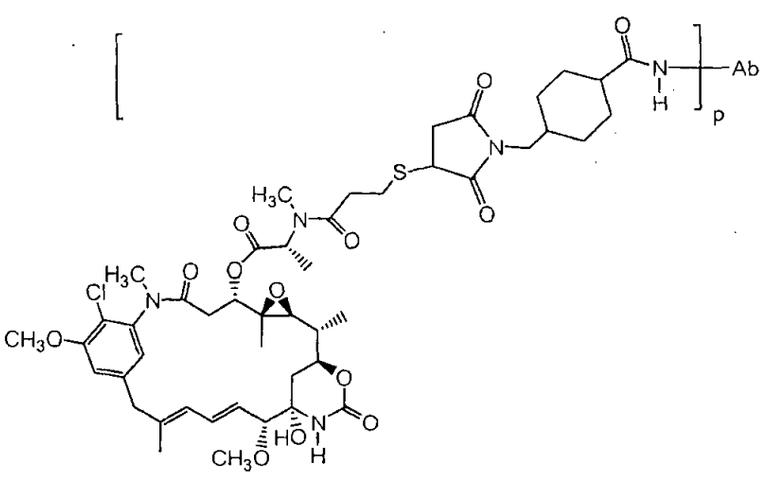
DM4

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, anti-HER2 antibody) 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):

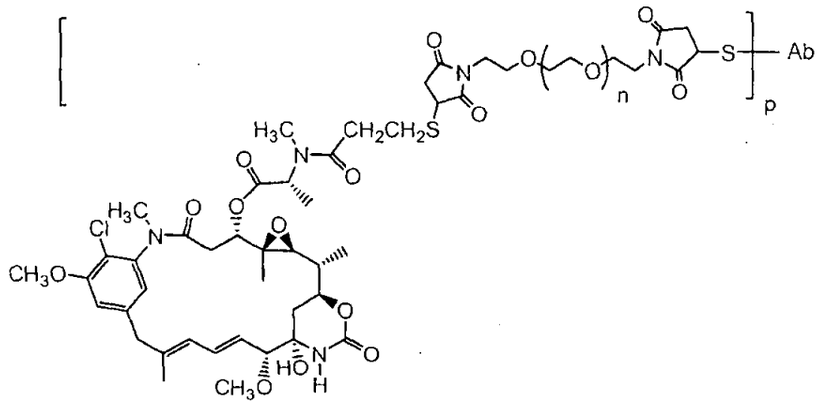


Ab -SPP-DM1



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.

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-CD22 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.

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.

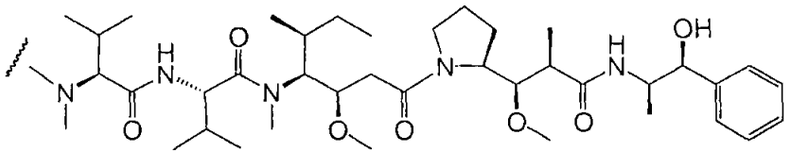
In one embodiment, any of the antibodies of the invention (full length or fragment) is conjugated to one or more maytansinoid molecules. In one embodiment of the immunoconjugate, the cytotoxic agent D, is a maytansinoid DM1. In one embodiment of the immunoconjugate, the linker is SMCC. In one embodiment, the antibody-linker-drug conjugate is an anti-CD22 antibody as disclosed herein to which is covalently DM1 cytotoxic agent via the SMCC linker.

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).

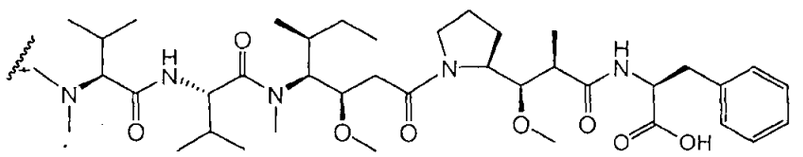
Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented March 28, 2004, the disclosure of which is expressly incorporated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



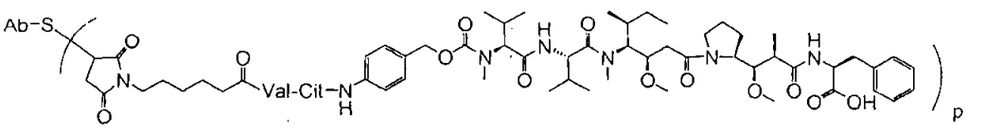
MMAE

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):

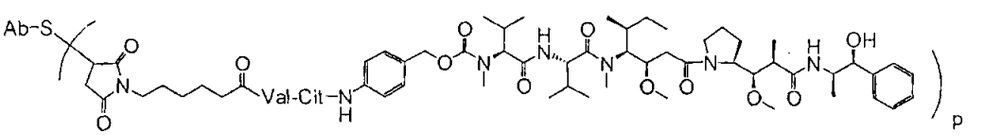


MMAF

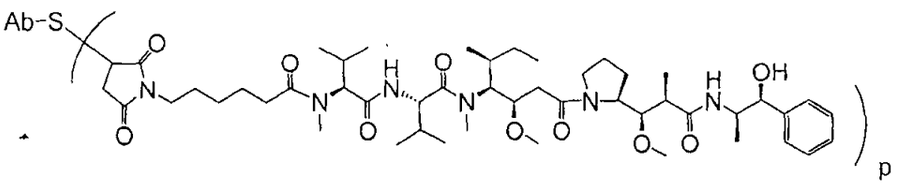
Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):



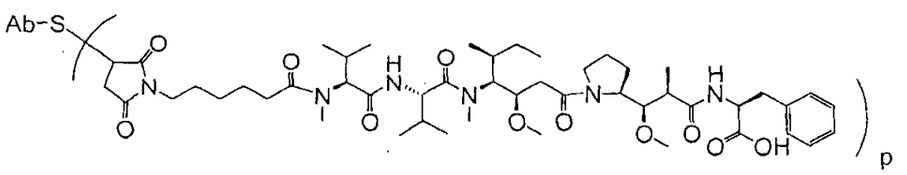
Ab-MC-vc-PAB-MMAF



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.

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 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:

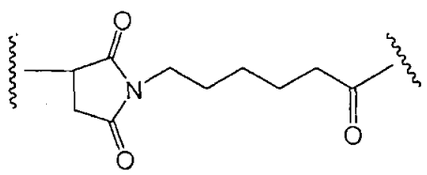
In the antibody drug conjugates (ADC) of the invention, an antibody (Ab) is conjugated to one or more drug moieties (D), e.g. about 1 to about 20 drug moieties per antibody, through a linker (L). The ADC of Formula I may be prepared by several routes, employing organic chemistry reactions, conditions, 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.

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

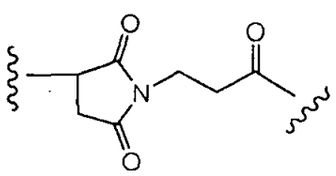
The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl ("PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC'), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB"). 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.

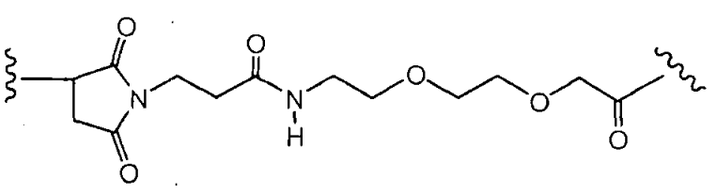
Exemplary linker component structures are shown below (wherein the wavy line indicates sites of covalent attachment to other components of the ADC):



MC

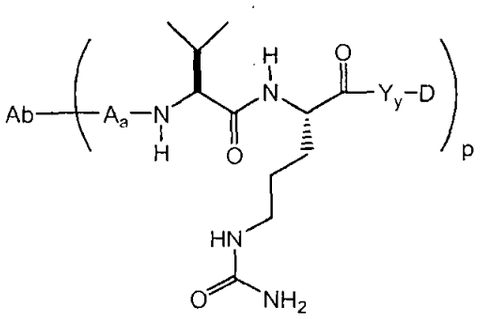


MP

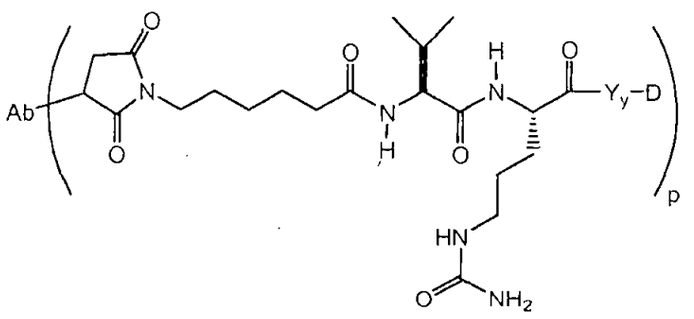


MPEG

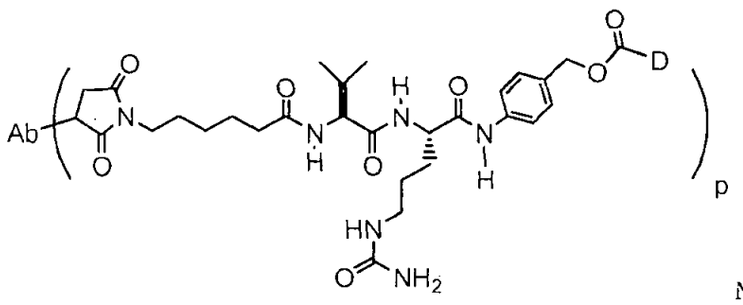
Additional exemplary linker components and abbreviations include (wherein the antibody (Ab) and linker are depicted, and p is 1 to about 8):



Val-cit



MC-val-cit



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 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.

In yet another aspect, the antibody has one or more lysine residues that can be chemically modified to introduce one or more sulfhydryl groups. The antibody unit bonds to the Linker unit via the sulfhydryl group's sulfur atom. The reagents that can be used to modify lysines include, but are not limited to, N-succinimidyl S-acetylthioacetate (SATA) and 2-Iminothiolane hydrochloride (Traut's Reagent).

In another embodiment, the antibody can have one or more carbohydrate groups that can be chemically modified to have one or more sulfhydryl groups. The antibody unit bonds to the Linker Unit, such as the Stretcher Unit, via the sulfhydryl group's sulfur atom, as disclosed herein.

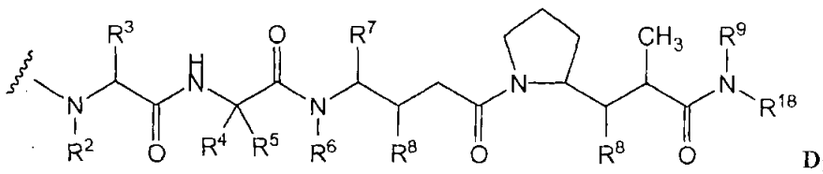
In yet another embodiment, the antibody can have one or more carbohydrate groups that can be oxidized to provide an aldehyde (-CHO) group (see, for e.g., Laguzza, et al., J. Med. Chem. 1989, 32(3), 548-55). The corresponding aldehyde can form a bond with a Reactive Site on a Stretcher. Reactive sites on a Stretcher that can react with a carbonyl group on an antibody include, but are not limited to, hydrazine and hydroxylamine. Other protocols for the modification of proteins for the attachment or association of Drug Units are described in Coligan et al., Current Protocols in Protein Science, vol. 2, John Wiley & Sons (2002), incorporated herein by reference.

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.

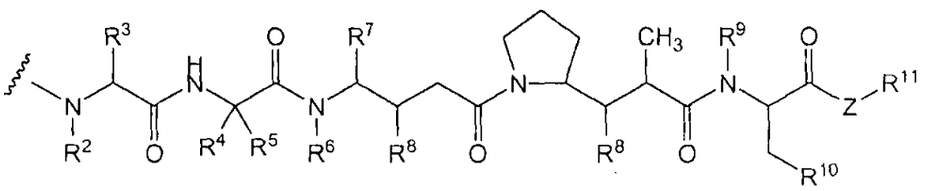
Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. The length of DNA may comprise respective regions encoding the two portions of the conjugate either adjacent one another or separated by a region encoding a linker 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



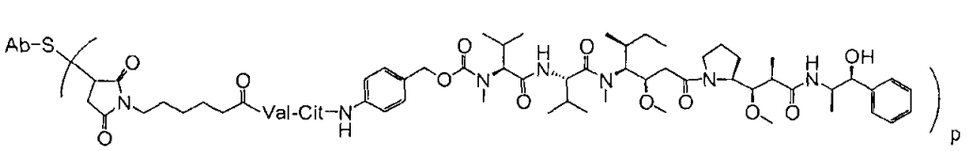
D_F

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

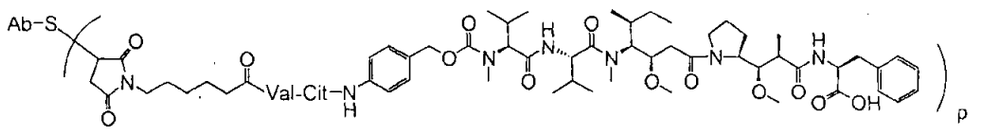
(d) p ranges from about 1 to 8.

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-CD22 antibodies, S is a sulfur atom, and p ranges from 2 to 5. In one embodiment, the immunoconjugate has the formula



wherein Ab is any of the above anti-CD22 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 B cell proliferative disorder is provided, wherein the method comprises administering to an individual the pharmaceutical composition. In one embodiment, the B cell proliferative disorder is selected from 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. In one embodiment, the cell proliferative disorder is associated with increased expression of CD22 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 CD22. In one embodiment, the B cell is a tumor cell. In one embodiment, the tumor cell is a B cell of a mammal experiencing or suspected of experiencing aa B cell proliferative disorder selected from 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., the cell 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-CD22 antibody of the invention is provided to assay serum soluble CD22 in a mammal experiencing leukemia or lymphoma to diagnose B-cell leukemia or B-cell lymphoma, measuring clinical progression or regression of the diseases, or assess tumor burden or relapse. Such methods are disclosed in US 20050244828 (Kreitman, R.J. et al., the entire contents of which is hereby incorporated by

reference) using an anti-CD22 RFB4 antibody PE38 (Pseudomonas exotoxin A fragment 38) toxin conjugate (see Kreitman, R.J. et al., NEJM 345:241-247 (2001)).

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1D: **Figure 1A** is a diagram of CD22 indicating the seven immunoglobulin-like domains of the extracellular domain of the beta isoform. The alpha isoform lacks domains 3 and 4. "TM" refers to transmembrane domain. **Figure 1B** depicts the amino acid sequence of the beta form of CD22 (SEQ ID NO:27). The alpha form of CD22 lacks the amino acids shown in italics (encoding domains 3 and 4 of the extracellular domain). The extracellular domain of the mature form of the protein is underlined (SEQ ID NO:28). Amino acids 1-21 depict the signal sequence cleaved from the mature form. **Figure 1C** is the amino acid sequence of CD22alpha (SEQ ID NO:29). The ECD of CD22alpha is underlined (SEQ ID NO:30). **Figure 1D** is the amino acid sequence of CD22 from cynomolgus monkey (cyno) (SEQ ID NO:31). The first 19 amino acids of cyno CD22 is the signal sequence.

Figures 2A-2B: **Figure 2A** depicts the amino acid sequence of the heavy chain variable region of murine 10F4 anti-CD22 antibody of the invention (m10F4) aligned with the humanized 10F4 version 1 antibody (h10F4v1) and aligned with the human subgroup III sequence. The HVRs are boxed (HVR-H1, HVR-H2, HVR-H3). The sequences bracketing the HVRs are the framework sequences (FR-H1 to FR-H4). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed HVRs. **Figure 2B** depicts the amino acid sequence of the light chain variable region of murine 10F4 anti-CD22 antibody of the invention (m10F4) aligned with the humanized 10F4 version 1 antibody (h10F4v1) and aligned with the human kappa I sequence. Versions 2 and 3 of the humanized 10F4 antibody (h10F4v2 and h10F4v3) have the same amino acid sequences for the secreted mature form. The antibodies h10F4v2 and h10F4v3 differ from h10F4v1 at amino acid 28 of the HVR-L1 (N28V). The HVRs are boxed. The FR-L1, FR-L2, FR-L3, and FR-L4 sequences bracket the HVRs (HVR-L1, HVR-L2, HVR-L3). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed HVRs.

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, 47, 48, 7).

- human VH subgroup I consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:50, 51, 52, 7; SEQ ID NOs:50, 51, 52, 7; and SEQ ID NOs:50, 51, 53, 7).

- human VH subgroup II consensus framework "A" minus Kabat CDRs (SEQ ID NOs:54, 55, 56, 7).

- human VH subgroup II consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:57, 58, 56, 7; SEQ ID NOs:57, 58, 59, 7; and SEQ ID NOs:57, 58, 60, 7).

- human VH subgroup III consensus framework "A" minus Kabat CDRs (SEQ ID NOs:61, 62, 63, 7).

- human VH subgroup III consensus frameworks "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:64, 65, 63, 7; SEQ ID NOs:64, 65, 66, 7; and SEQ ID NOs:64, 65, 67, 7).

- human VH acceptor 1 framework "A" minus Kabat CDRs (SEQ ID NOs:68, 62, 69, 7).

- human VH acceptor frameworks "B" and "C" minus extended hypervariable regions (SEQ ID NOs:64, 65, 69, 7; and SEQ ID NOs:64, 65, 70, 7).

- human VH acceptor 2 framework "A" minus Kabat CDRs (SEQ ID NOs:68, 62, 71, 7).

- human VH acceptor 2 framework "B," "C," and "D" minus extended hypervariable regions (SEQ ID NOs:64, 65, 71, 7; SEQ ID NOs:64, 65, 72, 7; and SEQ ID NOs:64, 65, 73, 7).

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:74, 75, 76, 77

- human VL kappa subgroup I consensus framework (κv1): SEQ ID NOs:74, 78, 76, 77

- human VL kappa subgroup II consensus framework (κv2): SEQ ID NOs:49, 79, 80, 77

- human VL kappa subgroup III consensus framework (κv3): SEQ ID NOs:81, 82, 83, 77

- human VL kappa subgroup IV consensus framework (κv4): SEQ ID NOs:84, 85, 86, 77

Figures 5A and 5B: Figure 5A depicts alignments of native sequence human IgG Fc region sequences, humIgG1 (non-A allotype, SEQ ID NO:38; and A allotype, where the amino acid sequence SREEM within SEQ ID NO:38 is changed to SRDEL), humIgG2 (SEQ ID NO:39), humIgG3 (SEQ ID NO:40) and humIgG4 (SEQ ID NO:41) 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. **Figure 5B** depicts the full length amino acid sequences (variable and constant regions) of the light and heavy chains of humanized anti-CD22 antibody 10F4v2, isotype IgG1. The underlined portions depict the constant domains.

Figures 6A-6D show results of assays measuring various determinants of CD22 ADC efficacy in lymphoma cell lines. **Figure 6A** indicates that higher cell surface CD22 levels are correlated to a lower anti-CD22-MCC-DM1 IC50 (higher efficacy). **Figure 6B**

indicates that increased internalization of anti-CD22-MCC-DM1 correlates with lower anti-CD22-MCC-DM1 IC50. **Figure 6C** indicates that increased intrinsic sensitivity of cells to free drug correlates with lower anti-CD22-MCC-DM1 IC50. **Figure 6D** is a photomicrograph showing the internalization of fluorescently labeled anti-CD22 antibody following binding to CD22 on the cell surface.

Figures 7A - 7B: **Figure 7A** is a graph of in vivo tumor volume reduction in a xenograft model which shows that administration of anti-CD22 antibody mu10F4-smcc-DM1 and hu10F4v1-smcc-DM1 to SCID mice having human B cell tumors significantly reduced tumor volume. Drug load was approximately 4 and 4.6, see Table 4. **Figure 7B** is a graph of a similar study, but drug load was slightly lower at approximately 2.9 and 3.0 (see Table 5), and mu10F4-smcc-DM1 and hu10F4v2-smcc-DM1 efficacy were compared with control antibody and unconjugated mu10F4. **Figure 7C** is a graph of in vivo tumor reduction in a xenograft model in which anti-CD22-spp-DM1 was administered as indicated in Table 6.

Figures 8A and 8B: **Figure 8A** is a graph of anti-CD22 antibodies 5E8.1.8-smcc-DM1 and RFB4-smcc-DM1 administered to Ramos cell xenografts. **Figure 8B** is a graph of anti-CD22 antibodies 5E8.1.8-smcc-DM1 and RFB4-smcc-DM1 administered to BJAB-luc xenografts.

Figure 9 is a graph showing the relative affect on tumor volume over time after administration of anti-CD22(RFB4)-smcc-DM1 at low, medium, and high drug loads.

Figure 10 is a graph showing the relative affect on tumor volume over time after administration of anti-CD22(RFB4)-MC-vcPAB-MMAF or anti-CD22(RFB4)-MC-MMAF in Ramos xenografts.

Figure 11 is a graph showing the relative affect on tumor volume over time after administration of anti-CD22(RFB4)-smcc-DM1 or -MCvcPAB-MMAE.

Figure 12 is a graph showing the relative affect on tumor volume over time after administration of humanized anti-CD22 10F4 variants as MMAF or DM1 immunoconjugates as disclosed in Table 12.

Figures 13A-13C are graphs showing the relative affect on tumor volume over time after administration anti-CD22-smcc-DM1 or anti-CD22-MC-MMAF in different B cell lymphoma xenograft models: SuDHL-4 (Figure 13A), DoHH2 (Figure 13B), and Granta-519 (Figure 13C).

Figure 14 shows diagrams of CD22 domains deleted for epitope mapping as described in the Examples. The domains are numbered 1-7. "TM" refers to transmembrane domain.

Figure 15 shows depictions of cysteine engineered anti-CD22 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-CD22 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-CD22 antibody drug conjugate (ADC).

Figures 17A-17C depict the amino acid sequences of the anti-CD22 cysteine engineered antibodies of the invention in which the light chain or heavy chain or Fc region is altered to engineer a cysteine at selected amino acid positions. Figure 17A depicts the amino acid sequence of the anti-CD22 10F4 variant light chain in which a valine at Kabat position 205 (sequential position Valine 210) is altered to a Cysteine. Figure 17B depicts the amino acid sequence of the anti-CD22 10F4 variant heavy chain in which an Alanine at EU position 118 (sequential position Alanine 121) is altered to a Cysteine. Figure 17C depicts the amino acid sequence of the anti-CD22 10F4 variant Fc region in which a Serine at EU position 400 (sequential position Serine 403) is altered to a Cysteine. In each figure, the altered amino acid is shown in bold text with double underlining. Single underlining indicates constant regions. Variable regions are not underlined.

Figures 18A-18E are FACS plots indicating that binding of anti-CD22 thiomab drug conjugates (TDCs) of the invention bind to CD22 expressed on the surface of BJAB-

lucs cells is similar for LC, HC and Fc thiomab variants as well as for the different drug conjugates shown.

Figure 19 is a graph plotting changes in mean tumor volume over time in a xenograft model treated with different anti-CD22 TDCs, which varied by position of the engineered cysteine (LC, HC or Fc) and/or by drug conjugate (MMAF or MMAE). Xenograft models treated with anti-CD22 TDCs 10F4-LC-V210C-MCvcPAB-MMAE and anti-CD22 10F4-HC-A121C-MCvcPAB-MMAE showed a decrease in tumor volume during the study.

Figure 20A is a graph plotting changes in mean tumor volume over time in a human mantle cell lymphoma Granta-519 xenograft in CB17 SCID mice treated with heavy chain A118C anti-CD22 TDCs conjugated to different linker drug moieties and/or administered at different doses as shown. The anti-CD22 10F4-HC(A118C)-MCvcPAB-MMAE TDC appeared to be the most efficacious of the test agents in this experiment. **Figure 20B** is a graph plotting changes in mean tumor volume over time in a follicular lymphoma DOHH2 xenograft in CB17 SCID mice treated with the same heavy chain A118C anti-CD22 TDCs, but at higher doses. The anti-CD22 10F4-HC(A118C)-MCvcPAB-MMAE TDC appeared to be the most efficacious of the test agents in this experiment. **Figure 20C** is a plot of percent weight change is the mice from the DOHH2 xenograft study showing that there was no significant change in weight during the first 14 days of the study.

Figures 21A and 21B are bar graphs showing changes in serum AST (aspartate aminotransferase) (**Figure 21A**) and serum neutrophils (**Figure 21B**) at Days 0 and 5 where ADC comprising a cleavable and uncleavable linker was administered.

Figures 22A and 22B are graphs showing depletion of peripheral B cells ($CD20^+$ cells) in cynomolgus monkeys dosed with 10, 20, and 30 mg/kg anti-CD22 MMAF (**Figure 22A**) and anti-CD22 DM1 (**Figure 22B**).

Figures 23A and 23B are graphs showing no significant change in $CD4^+$ lymphocytes at 10, 20, and 30 mg/kg anti- CD22 MMAF (**Figure 23A**) and anti-CD22 DM1 (**Figure 23B**).

Figures 24A and 24B show histological samples of cynomolgus monkey tonsil tissue in which depletion of germinal center B cells, apparent in the vehicle control (**Figure 24A**), are depleted in a tonsil sample from an animal dosed at 10 mg/kg hu10F4v3-SMCC-DM1.

Figure 25A is a diagram indicating the regions of spleen follicle from which tissue samples were taken for a study in which it was shown that anti-CD22 ADCs spare B cells in resting tissue in cynomolgus monkeys. Dividing cells in cyno spleen follicle germinal center were depleted in dividing germinal cells of cyno spleens from animals dosed with hu10F4v3-MC-MMAF at 10 mg/kg (**Figures 25B and 25C**). Non-dividing naïve B cells were not depleted under the same conditions (**Figures 25D and 25E**).

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Isolated antibodies that bind to CD22 are provided. Immunoconjugates comprising anti-CD22 antibodies are further provided. Cysteine engineered anti-CD22 antibodies and immunoconjugates thereof 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 CD22. 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, antibodies or immunoconjugates of the invention are useful for the detection of CD22, e.g., CD22 expressed on the cell surface.

Polynucleotides encoding anti-CD22 antibodies are provided. Vectors comprising polynucleotides encoding anti-CD22 antibodies are provided, and host cells comprising such vectors are provided. Compositions, including pharmaceutical formulations, comprising any one or more of the polynucleotides, anti-CD22 antibodies, or immunoconjugates of the invention are also provided.

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.): Per 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 homogencity 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). 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 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 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.

A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antagonist that binds thereto, including

but not limited to, antibodies to a B-cell surface antigen or a soluble form a B-cell surface antigen capable of antagonizing binding of a ligand to the naturally occurring B-cell antigen. Exemplary B-cell surface markers include the CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers (for descriptions, see The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay et al. Academic Press, Harcourt Brace & Co., New York). Other B-cell surface markers include RP105, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, BAFF, BLyS, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells.

The term "CD22," as used herein, refers to any native CD22 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 CD22 as well as any form of CD22 that results from processing in the cell. The term also encompasses naturally occurring variants of CD22, e.g., splice variants, allelic variants, and isoforms. The major isoform of CD22 (CD22beta) comprises 847 amino acids and seven immunoglobulin-like regions in the extracellular domain (see Wilson, G.L. et al., J. Exp. Med. 173:137-146 (1991)). A minor isoform, CD22alpha, comprises 647 amino acids and lacks immunoglobulin-like domains 3 and 4 in the extracellular domain (see Stamenkovic, I. and Seed, B., Nature 345:74-77 (1990)) and Wilson et al. (1991), supra). The amino acid sequence of CD22 beta is depicted in Figure 1B in which the underlined portion is the extracellular domain (ECD) and the italicized portion indicates the amino acids missing from the CD22 alpha extracellular domain sequence. Figure 1C depicts the amino acid sequence of CD22alpha in which the ECD is underlined. The amino acid sequence from amino acid 1 to amino acid 21 represents the signal sequence cleaved from the mature form of the protein. In one embodiment, CD22 is expressed on the cell surface, such as on the surface of a normal B cell or a tumor B cell. Figure 1D depicts the amino acid sequence of CD22 from cynomolgus monkey.

"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-CD22 antibody" or "an antibody that binds to CD22" refers to an antibody that is capable of binding CD22 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD22. Preferably, the extent of binding of an anti-CD22 antibody to an unrelated, non-CD22 protein is less than about 10% of the binding of the antibody to CD22 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD22 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-CD22 antibody binds to an epitope of CD22 that is conserved among CD22 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 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 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 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 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 of 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 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 (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 and HVR-H2 hypervariable regions of the anti-CD22 10F4 antibodies of the invention are H26-H35 and H49-H65 using Kabat numbering.

"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.

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 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-dioxaoctanediamine, 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.

The following abbreviations are used herein and have the indicated definitions: BME is beta-mercaptoethanol, Boc is N-(t-butoxycarbonyl), cit is citrulline (2-amino-5-ureido pentanoic acid), 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, PAB is p-aminobenzylcarbamoyl, PBS is phosphate-buffered saline (pH 7), 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.

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 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).

"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). 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:1)-HVR-H1-
WVRQAPGKGLEWV (FR-H2, SEQ ID NO:3)-HVR-H2-
RFTISADTSKNTAYLQMNSLRAEDTAVYYC (FR-H3, SEQ ID NO:5)-HVR-H3-
WGQGTLVTVSS (FR-H4, SEQ ID NO:7).

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:8)-HVR-L1-
WYQQKPGKAPKLLIY (FR-L2, SEQ ID NO:11)-HVR-L2-
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, SEQ ID NO:13)-HVR-L3-
FGQGTKVEIK (FR-L4, SEQ ID NO:15).

"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 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

"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/proliferation. Examples of cancer include, but are not limited to, cancerous B cell proliferative disorders B cell proliferative disorder is selected from 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. Other cancers conditions included, for example, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

A "B-cell malignancy" herein includes non-Hodgkin's lymphoma (NHL), including low grade/follicular NHL, small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia, non-Hodgkin's lymphoma (NHL), lymphocyte predominant Hodgkin's disease (LPHD), small lymphocytic lymphoma (SLL), chronic lymphocytic leukemia (CLL), indolent NHL including relapsed indolent NHL and rituximab-refractory indolent NHL; leukemia, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia, chronic myeloblastic leukemia; mantle cell lymphoma; and other hematologic malignancies. Such malignancies may be treated with antibodies directed against B-cell surface markers,

such as CD22. Such diseases are contemplated herein to be treated by the administration of an antibody directed against a B cell surface marker, such as CD22, and includes the administration of an unconjugated ("naked") antibody or an antibody conjugated to a cytotoxic agent as disclosed herein. Such diseases are also contemplated herein to be treated by combination therapy including an anti-CD22 antibody or anti-CD22 antibody drug conjugate of the invention in combination with another antibody or antibody drug conjugate, another cytoxic agent, radiation or other treatment administered simultaneously or in series. In exemplary treatment method of the invention, an anti-CD22 antibody of the invention is administered in combination with an anti-CD20 antibody, immunoglobulin, or CD20 binding fragment thereof, either together or sequentially. The anti-CD20 antibody may be a naked antibody or an antibody drug conjugate. In an embodiment of the combination therapy, the anti-CD22 antibody is an antibody of the present invention and the anti-CD20 antibody is Rituxan® (rituximab).

The term "non-Hodgkin's lymphoma" or "NHL", as used herein, refers to a cancer of the lymphatic system other than Hodgkin's lymphomas. Hodgkin's lymphomas can generally be distinguished from non-Hodgkin's lymphomas by the presence of Reed-Sternberg cells in Hodgkin's lymphomas and the absence of said cells in non-Hodgkin's lymphomas. Examples of non-Hodgkin's lymphomas encompassed by the term as used herein include any that would be identified as such by one skilled in the art (e.g., an oncologist or pathologist) in accordance with classification schemes known in the art, such as the Revised European-American Lymphoma (REAL) scheme as described in Color Atlas of Clinical Hematology (3rd edition), A. Victor Hoffbrand and John E. Pettit (eds.) (Harcourt Publishers Ltd., 2000). See, in particular, the lists in Fig. 11.57, 11.58 and 11.59. More specific examples include, but are not limited to, relapsed or refractory NHL, front line low grade NHL, Stage III/IV NHL, chemotherapy resistant NHL, precursor B lymphoblastic leukemia and/or lymphoma, small lymphocytic lymphoma, B cell chronic lymphocytic leukemia and/or prolymphocytic leukemia and/or small lymphocytic lymphoma, B-cell prolymphocytic lymphoma, immunocytoma and/or lymphoplasmacytic lymphoma, lymphoplasmacytic lymphoma, marginal zone B cell lymphoma, splenic marginal zone lymphoma, extranodal marginal zone - MALT lymphoma, nodal marginal zone lymphoma, hairy cell leukemia, plasmacytoma and/or plasma cell myeloma, low grade/follicular lymphoma, intermediate grade/follicular NHL, mantle cell lymphoma,

follicle center lymphoma (follicular), intermediate grade diffuse NHL, diffuse large B-cell lymphoma, aggressive NHL (including aggressive front-line NHL and aggressive relapsed NHL), NHL relapsing after or refractory to autologous stem cell transplantation, primary mediastinal large B-cell lymphoma, primary effusion lymphoma, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, Burkitt's lymphoma, precursor (peripheral) large granular lymphocytic leukemia, mycosis fungoides and/or Sezary syndrome, skin (cutaneous) lymphomas, anaplastic large cell lymphoma, angiocentric lymphoma.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. In many of these autoimmune and inflammatory disorders, a number of clinical and laboratory markers may exist, including, but not limited to, hypergammaglobulinemia, high levels of autoantibodies, antigen-antibody complex deposits in tissues, benefit from corticosteroid or immunosuppressive treatments, and lymphoid cell aggregates in affected tissues. Without being limited to any one theory regarding B-cell mediated autoimmune disease, it is believed that B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neo-lymphogenesis. Each of these pathways may participate to different degrees in the pathology of autoimmune diseases.

"Autoimmune disease" can be an organ-specific disease (i.e., the immune response is specifically directed against an organ system such as the endocrine system, the hematopoietic system, the skin, the cardiopulmonary system, the gastrointestinal and liver systems, the renal system, the thyroid, the ears, the neuromuscular system, the central nervous system, etc.) or a systemic disease which can affect multiple organ systems (for example, systemic lupus erythematosus (SLE), rheumatoid arthritis, polymyositis, etc.). Preferred such diseases include autoimmune rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease),

autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-negative vasculitis and ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and microscopic polyangiitis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (e.g., Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus, multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

Specific examples of other autoimmune diseases as defined herein, which in some cases encompass those listed above, include, but are not limited to, arthritis (acute and chronic, rheumatoid arthritis including juvenile-onset rheumatoid arthritis and stages such as rheumatoid synovitis, gout or gouty arthritis, acute immunological arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, osteoarthritis, arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, menopausal arthritis, estrogen-depletion arthritis, and ankylosing spondylitis/rheumatoid spondylitis), autoimmune lymphoproliferative disease, inflammatory hyperproliferative skin diseases, psoriasis such as plaque psoriasis, gutatte psoriasis, pustular psoriasis, and psoriasis of the nails, atopy including atopic diseases such as hay fever and Job's syndrome, dermatitis including contact dermatitis, chronic contact

dermatitis, exfoliative dermatitis, allergic dermatitis, allergic contact dermatitis, hives, dermatitis herpetiformis, nummular dermatitis, seborrheic dermatitis, non-specific dermatitis, primary irritant contact dermatitis, and atopic dermatitis, x-linked hyper IgM syndrome, allergic intraocular inflammatory diseases, urticaria such as chronic allergic urticaria and chronic idiopathic urticaria, including chronic autoimmune urticaria, myositis, polymyositis/dermatomyositis, juvenile dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as systemic sclerosis, multiple sclerosis (MS) such as spino-optical MS, primary progressive MS (PPMS), and relapsing remitting MS (RRMS), progressive systemic sclerosis, atherosclerosis, arteriosclerosis, sclerosis disseminata, ataxic sclerosis, neuromyelitis optica (NMO), inflammatory bowel disease (IBD) (for example, Crohn's disease, autoimmune-mediated gastrointestinal diseases, gastrointestinal inflammation, colitis such as ulcerative colitis, colitis ulcerosa, microscopic colitis, collagenous colitis, colitis polyposa, necrotizing enterocolitis, and transmural colitis, and autoimmune inflammatory bowel disease), bowel inflammation, pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, respiratory distress syndrome, including adult or acute respiratory distress syndrome (ARDS), meningitis, inflammation of all or part of the uvea, iritis, choroiditis, an autoimmune hematological disorder, graft-versus-host disease, angioedema such as hereditary angioedema, cranial nerve damage as in meningitis, herpes gestationis, pemphigoid gestationis, pruritis scroti, autoimmune premature ovarian failure, sudden hearing loss due to an autoimmune condition, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis and limbic and/or brainstem encephalitis, uveitis, such as anterior uveitis, acute anterior uveitis, granulomatous uveitis, nongranulomatous uveitis, phacoantigenic uveitis, posterior uveitis, or autoimmune uveitis, glomerulonephritis (GN) with and without nephrotic syndrome such as chronic or acute glomerulonephritis such as primary GN, immune-mediated GN, membranous GN (membranous nephropathy), idiopathic membranous GN or idiopathic membranous nephropathy, membrano- or membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN (RPGN), proliferative nephritis, autoimmune polyglandular endocrine failure, balanitis including balanitis circumscripta plasmacellularis, balanoposthitis, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiform, granuloma annulare, lichen nitidus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, lichen planus, lamellar ichthyosis, epidermolytic

hyperkeratosis, premalignant keratosis, pyoderma gangrenosum, allergic conditions and responses, food allergies, drug allergies, insect allergies, rare allergic disorders such as mastocytosis, allergic reaction, eczema including allergic or atopic eczema, asteatotic eczema, dyshidrotic eczema, and vesicular palmoplantar eczema, asthma such as asthma bronchiale, bronchial asthma, and auto-immune asthma, conditions involving infiltration of T cells and chronic inflammatory responses, immune reactions against foreign antigens such as fetal A-B-O blood groups during pregnancy, chronic pulmonary inflammatory disease, autoimmune myocarditis, leukocyte adhesion deficiency, lupus, including lupus nephritis, lupus cerebritis, pediatric lupus, non-renal lupus, extra-renal lupus, discoid lupus and discoid lupus erythematosus, alopecia lupus, SLE, such as cutaneous SLE or subacute cutaneous SLE, neonatal lupus syndrome (NLE), and lupus erythematosus disseminatus, juvenile onset (Type I) diabetes mellitus, including pediatric IDDM, adult onset diabetes mellitus (Type II diabetes), autoimmune diabetes, idiopathic diabetes insipidus, diabetic retinopathy, diabetic nephropathy, diabetic colitis, diabetic large-artery disorder, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, agranulocytosis, vasculitides (including large-vessel vasculitis such as polymyalgia rheumatica and giant-cell (Takayasu's) arteritis, medium-vessel vasculitis such as Kawasaki's disease and polyarteritis nodosa/periarteritis nodosa, immunovasculitis, CNS vasculitis, cutaneous vasculitis, hypersensitivity vasculitis, necrotizing vasculitis such as fibrinoid necrotizing vasculitis and systemic necrotizing vasculitis, ANCA-negative vasculitis, and ANCA-associated vasculitis such as Churg-Strauss syndrome (CSS), Wegener's granulomatosis, and microscopic polyangiitis), temporal arteritis, aplastic anemia, autoimmune aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia (anemia perniciosa), Addison's disease, pure red cell anemia or aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia(s), cytopenias such as pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, Alzheimer's disease, Parkinson's disease, multiple organ injury syndrome such as those secondary to septicemia, trauma or hemorrhage, antigen-antibody complex- mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, motoneuritis, allergic neuritis, Behçet's disease/syndrome, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjögren's syndrome,

Stevens-Johnson syndrome, pemphigoid or pemphigus such as pemphigoid bullous, cicatricial (mucous membrane) pemphigoid, skin pemphigoid, pemphigus vulgaris, paraneoplastic pemphigus, pemphigus foliaceus, pemphigus mucus-membrane pemphigoid, and pemphigus erythematosus, epidermolysis bullosa acquisita, ocular inflammation, preferably allergic ocular inflammation such as allergic conjunctivis, linear IgA bullous disease, autoimmune-induced conjunctival inflammation, autoimmune polyendocrinopathies, Reiter's disease or syndrome, thermal injury due to an autoimmune condition, preeclampsia, an immune complex disorder such as immune complex nephritis, antibody-mediated nephritis, neuroinflammatory disorders, polyneuropathies, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP), post-transfusion purpura (PTP), heparin-induced thrombocytopenia, and autoimmune or immune-mediated thrombocytopenia including, for example, idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, scleritis such as idiopathic cerato-scleritis, episcleritis, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, Hashimoto's disease, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Grave's disease, Grave's eye disease (ophthalmopathy or thyroid-associated ophthalmopathy), polyglandular syndromes such as autoimmune polyglandular syndromes, for example, type I (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis or encephalomyelitis allergica and experimental allergic encephalomyelitis (EAE), myasthenia gravis such as thymoma-associated myasthenia gravis, cerebellar degeneration, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, multifocal motor neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, giant-cell hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, pneumonitis such as lymphoid interstitial pneumonitis (LIP), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), idiopathic IgA nephropathy, linear IgA dermatosis, acute febrile neutrophilic dermatosis, subcorneal pustular dermatosis,

transient acantholytic dermatosis, cirrhosis such as primary biliary cirrhosis and pneumonocirrhosis, autoimmune enteropathy syndrome, Celiac or Coeliac disease, celiac sprue (gluten enteropathy), refractory sprue, idiopathic sprue, cryoglobulinemia such as mixed cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune ear disease such as autoimmune inner ear disease (AIED), autoimmune hearing loss, polychondritis such as refractory or relapsed or relapsing polychondritis, pulmonary alveolar proteinosis, keratitis such as Cogan's syndrome/nonsyphilitic interstitial keratitis, Bell's palsy, Sweet's disease/syndrome, rosacea autoimmune, zoster-associated pain, amyloidosis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal or segmental or focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, chorioretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases such as autoimmune demyelinating diseases and chronic inflammatory demyelinating polyneuropathy, Dressler's syndrome, alopecia areata, alopecia totalis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, e.g., due to anti-spermatozoan antibodies, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, allergic granulomatous angiitis, benign lymphocytic angiitis, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, parasitic diseases such as leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, diffuse interstitial pulmonary fibrosis, interstitial lung fibrosis, fibrosing mediastinitis, pulmonary fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, iridocyclitis (acute or chronic), or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus

(HIV) infection, SCID, acquired immune deficiency syndrome (AIDS), echovirus infection, sepsis (systemic inflammatory response syndrome (SIRS)), endotoxemia, pancreatitis, thyroxicosis, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, chorioiditis, giant-cell polymyalgia, chronic hypersensitivity pneumonitis, conjunctivitis, such as vernal catarrh, keratoconjunctivitis sicca, and epidemic keratoconjunctivitis, idiopathic nephritic syndrome, minimal change nephropathy, benign familial and ischemia-reperfusion injury, transplant organ reperfusion, retinal autoimmunity, joint inflammation, bronchitis, chronic obstructive airway/pulmonary disease, silicosis, aphthae, aphthous stomatitis, arteriosclerotic disorders (cerebral vascular insufficiency) such as arteriosclerotic encephalopathy and arteriosclerotic retinopathy, aspermiogenese, autoimmune hemolysis, Boeck's disease, cryoglobulinemia, Dupuytren's contracture, endophthalmia phacoanaphylactica, enteritis allergica, erythema nodosum leprosum, idiopathic facial paralysis, chronic fatigue syndrome, febris rheumatica, Hamman-Rich's disease, sensoneural hearing loss, haemoglobinuria paroxysmatica, hypogonadism, ileitis regionalis, leucopenia, mononucleosis infectiosa, traverse myelitis, primary idiopathic myxedema, nephrosis, ophthalmia symphatica (sympathetic ophthalmitis), neonatal ophthalmitis, optic neuritis, orchitis granulomatosa, pancreatitis, polyradiculitis acuta, pyoderma gangrenosum, Quervain's thyreoiditis, acquired spenic atrophy, non-malignant thymoma, lymphofollicular thymitis, vitiligo, toxic-shock syndrome, food poisoning, conditions involving infiltration of T cells, leukocyte-adhesion deficiency, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, diseases involving leukocyte diapedesis, multiple organ injury syndrome, antigen-antibody complex-mediated diseases, antiglomerular basement membrane disease, autoimmune polyendocrinopathies, oophoritis, primary myxedema, autoimmune atrophic gastritis, rheumatic diseases, mixed connective tissue disease, nephrotic syndrome, insulitis, polyendocrine failure, autoimmune polyglandular syndromes, including polyglandular syndrome type I, adult-onset idiopathic hypoparathyroidism (AOIH), cardiomyopathy such as dilated cardiomyopathy, epidermolisis bullosa acquisita (EBA), hemochromatosis, myocarditis, nephrotic syndrome, primary sclerosing cholangitis, purulent or nonpurulent sinusitis, acute or chronic sinusitis, ethmoid, frontal, maxillary, or sphenoid sinusitis, allergic sinusitis, an eosinophil-related disorder such as eosinophilia,

pulmonary infiltration eosinophilia, eosinophilia-myalgia syndrome, Loffler's syndrome, chronic eosinophilic pneumonia, tropical pulmonary eosinophilia, bronchopneumonic aspergillosis, aspergilloma, or granulomas containing eosinophils, anaphylaxis, spondyloarthropathies, seronegative spondyloarthritides, polyendocrine autoimmune disease, sclerosing cholangitis, sclera, episclera, chronic mucocutaneous candidiasis, Bruton's syndrome, transient hypogammaglobulinemia of infancy, Wiskott-Aldrich syndrome, ataxia telangiectasia syndrome, angiectasis, autoimmune disorders associated with collagen disease, rheumatism such as chronic arthrorheumatism, lymphadenitis, reduction in blood pressure response, vascular dysfunction, tissue injury, cardiovascular ischemia, hyperalgesia, renal ischemia, cerebral ischemia, and disease accompanying vascularization, allergic hypersensitivity disorders, glomerulonephritides, reperfusion injury, ischemic re-perfusion disorder, reperfusion injury of myocardial or other tissues, lymphomatous tracheobronchitis, inflammatory dermatoses, dermatoses with acute inflammatory components, multiple organ failure, bullous diseases, renal cortical necrosis, acute purulent meningitis or other central nervous system inflammatory disorders, ocular and orbital inflammatory disorders, granulocyte transfusion-associated syndromes, cytokine-induced toxicity, narcolepsy, acute serious inflammation, chronic intractable inflammation, pyelitis, endarterial hyperplasia, peptic ulcer, valvulitis, and endometriosis. Such diseases are contemplated herein to be treated by the administration of an antibody which binds to a B cell surface marker, such as CD22, and includes the administration of an unconjugated ("naked") antibody or an antibody conjugated to a cytotoxic agent as disclosed herein. Such diseases are also contemplated herein to be treated by combination therapy including an anti-CD22 antibody or anti-CD22 antibody drug conjugate of the invention in combination with another antibody or antibody drug conjugate, another cytoxic agent, radiation or other treatment administered simultaneously or in series.

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

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 BIAcoreTM-2000 or a BIAcoreTM-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 B cell proliferative disorders and/or B cell tumors, e.g., 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.

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.

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.

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.

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 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 omega1I (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 (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 triptorelin; 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 CD22) 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 CD22) 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 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', -OG(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, -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 -$(CH_2)_{1-10}$-. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene and decalene.

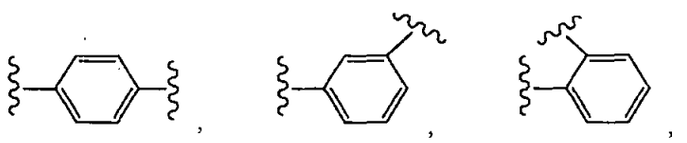
"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene. Typical alkenylene radicals include, 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:



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, $-C(=O)NR_2$, $-SO_3^-$, $-SO_3H$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, -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, 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', -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.

"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 (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 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 CD22 are provided. Immunoconjugates comprising anti-CD22 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 CD22. 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-CD22 Antibodies

In one aspect, the invention provides antibodies that bind to CD22. In some embodiments, antibodies are provided that bind to a mature form of human and cynomolgus monkey (cyno) CD22. In one such embodiment, a mature form of human CD22 has an amino acid sequence of SEQ ID NO:27. The mature, major human isoform has an extracellular domain comprising seven Ig-like domains and an amino acid sequence of SEQ ID NO:28. In another embodiment, a minor isoform of human CD22 lacking extracellular domains 3 and 4 has an amino acid sequence of SEQ ID NO: 29. The amino acid sequence of the extracellular domain of the minor isoform is SEQ ID NO:30. The cyno CD22 has an amino acid sequence of SEQ ID NO:31. In some embodiments, an antibody to CD22 binds to a mature form of CD22 expressed on the cell surface. In some embodiments, an antibody that binds to a mature form of CD22 expressed on the cell surface inhibits the growth of the cell. In some embodiments, an anti-CD22 antibody binds to a mature form of CD22 expressed on the cell surface and inhibits cell proliferation. In certain embodiments, an anti-CD22 antibody binds to a mature form of CD22 expressed on the cell surface and induces cell death. In some embodiments, an anti-CD22 antibody binds to a mature form of CD22 expressed on the surface of cancer cells. In some embodiments, an anti-CD22 antibody binds to a mature form of CD22 that is overexpressed on the surface of cancer cells relative to normal cells of the same tissue origin. In some embodiments, an anti-CD22 antibody is conjugated to a cytotoxin or a detectable label and binds to CD22 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 CD22 on its surface to be detectable in vitro or in vivo.

In one aspect, an anti-CD22 antibody is a monoclonal antibody. In one aspect, an anti-CD22 antibody is an antibody fragment, e.g., a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an anti-CD22 antibody is a chimeric, humanized, or human antibody. In one aspect, any of the anti-CD22 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 CD22 beta and alpha. The antibodies resulting from the library screen are affinity matured.

In one aspect, monoclonal antibodies that compete with murine 10F4.4.1, humanized 10F4v1 and v3, and murine 5E8.1.8 for binding to CD22 are provided. Monoclonal antibodies that bind to the same epitope as murine 10F4.4.1, humanized 10F4v1 and v3, and murine 5E8.1.8 are also provided.

In one aspect of the invention, polynucleotides encoding anti-CD22 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-CD22 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the invention, compositions comprising anti-CD22 antibodies or polynucleotides encoding anti-CD22 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.

Antibody Administration and Formulation

In one embodiment, the anti-CD22 antibody or anti-CD22 antibody drug conjugate (including, but not limited to, an anti-CD22 thiomab drug conjugate of the invention) of the invention is administered in combination with an antagonist of a B-cell surface antigen. Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order. In one embodiment, the administration is consecutive or sequential. In another embodiment, the administration is simultaneous, concurrent, or together in the same formulation. In one embodiment, the B-cell surface antigen antagonist is an antibody or antigen binding fragment thereof. In one embodiment, the B-cell surface antagonist is an antibody drug conjugate.

The formulations herein may contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to an anti-CD22 antibody, anti-CD22 antibody drug conjugate or CD22 binding oligopeptide, it may be desirable to include in the one formulation, an additional antibody, e.g., a second anti-CD22 antibody which binds a different epitope on the CD22 polypeptide, or a second antibody that binds a different B-cell surface antigen, or an antibody to some other target such as a growth factor that affects the growth of the particular cancer. Alternatively, or additionally, the composition may further comprise 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.

Currently, depending on the stage of the cancer, cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, and chemotherapy. Anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide therapy may be especially desirable in elderly patients who do not tolerate the toxicity and side effects of chemotherapy well and in metastatic disease where radiation therapy has limited usefulness. The tumor targeting anti-CD22 antibodies, anti-CD22 antibody drug conjugate or oligopeptide of the invention are useful to alleviate CD22-expressing cancers upon initial diagnosis of the disease or during relapse. For therapeutic applications, the anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide can be used alone, or in combination therapy with, e.g., hormones, antiangiogens, or radiolabelled compounds, or with surgery, cryotherapy, and/or radiotherapy. Anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide treatment can be administered in conjunction with other forms of conventional therapy, either consecutively with, pre- or post-conventional therapy. In the present method of the invention for treating or alleviating cancer, the cancer patient can be administered anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide in conjuction with treatment with the one or more of the preceding chemotherapeutic agents. The anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide will be administered with a therapeutically effective dose of the chemotherapeutic agent. In another embodiment, the anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide is administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent. The Physicians' Desk

Reference (PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill in the art and can be determined by the physician.

In one particular embodiment, a conjugate comprising an anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide conjugated with a cytotoxic agent is administered to the patient. Preferably, the immunoconjugate bound to the CD22 protein is internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In one embodiment, the cytotoxic agent targets or interferes with the nucleic acid in the cancer cell. Examples of cytotoxic agents are described above and include auristatins, maytansinoids, calicheamicins, ribonucleases and DNA endonucleases, or biologically active derivatives thereof.

The anti-CD22 antibodies, anti-CD22 antibody drug conjugates or oligopeptides or toxin conjugates thereof are administered to a human patient, in accord with known methods, such as intravenous administration, e.g.,, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody, anti-CD22 antibody drug conjugate or oligopeptide is preferred.

Other therapeutic regimens may be combined with the administration of the anti-CD22 antibody, anti-CD22 antibody drug conjugate or oligopeptide. The combined administration includes co-administration, 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. Preferably such combined therapy results in a synergistic therapeutic effect.

It may also be desirable to combine administration of the anti-CD22 antibody or antibodies, anti-CD22 antibody drug conjugates or oligopeptides, with administration of an

antibody directed against another tumor antigen or B-cell surface antigen associated with the particular cancer.

In another embodiment, the therapeutic treatment methods of the present invention involves the combined administration of an anti-CD22 antibody (or antibodies) , anti-CD22 antibody drug conjugate(s) or oligopeptide(s) and one or more chemotherapeutic agents or growth inhibitory agents, including co-administration of cocktails of different chemotherapeutic agents. Chemotherapeutic agents include estramustine phosphate, prednimustine, cisplatin, 5-fluorouracil, melphalan, cyclophosphamide, hydroxyurea and hydroxyureataxanes (such as paclitaxel and doxetaxel) and/or anthracycline antibiotics, as well as combinations of agents such as, but not limited to, CHOP or FOLFOX. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

The antibody is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, e.g., US Patent Appln No. 2002/0009444, Grillo-Lopez, A, concerning intrathecal delivery of a CD20 antibody). Preferably, the dosing is given intravenously or subcutaneously.

A second medicament may be administered with the initial exposure and/or later exposures of the therapeutic antibody or immunoadhesin, such combined administration includes co-administration, 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.

While the therapeutic anti-CD22 antibody, anti-CD22 antibody drug conjugate, immunoadhesin or other biologic may be administered as a single-agent to treat the autoimmune disease, generally, the therapeutic antibody or immunoadhesin will be combined with one or more second medicament(s). For example, for RA, and other

autoimmune diseases, the antibody, immunoadhesin, or other biologic drug is preferably combined with any one or more of the immunosuppressive agents, chemotherapeutic agents, BAFF antagonists, integrin antagonists or antibodies, and/or cytokines listed in the definitions section above; any one or more disease-modifying antirheumatic drugs (DMARDs), such as hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine; Staphylococcal protein A immunoadsorption; intravenous immunoglobulin (IVIG); nonsteroidal antiinflammatory drugs (NSAIDs); glucocorticoid (e.g. via joint injection); corticosteroid (e.g. methylprednisolone and/or prednisone); folate; an anti-tumor necrosis factor (TNF) antagonist, e.g. etanercept/ENBREL™, infliximab/REMICADE™, D2E7 (Knoll) or CDP-870 (Celltech); IL-1R antagonist (e.g. Kineret); IL-10 antagonist (e.g. Ilodecakin); a blood clotting modulator (e.g. WinRho); an IL-6 antagonist/anti-TNF (CBP 1011); CD40 antagonist (e.g. IDEC 131); Ig-Fc receptor antagonist (MDX33); immunomodulator (e.g. thalidomide or ImmuDyn); anti-CD5 antibody (e.g. H5g1.1); macrophage inhibitor (e.g. MDX 33); costimulatory blocker (e.g. BMS 188667 or Tolerimab); complement inhibitor (e.g. h5G1.1, 3E10 or an anti-decay accelerating factor (DAF) antibody); IL-2 antagonist (zxSMART); EGFR inhibitor (see definition above); tyrosine kinase inhibitor (see definition above); anti-angiogenic agent (e.g. VEGF antibody such as bevacizumab); CD22 antibodies such as LL2 or epratuzumab (LYMPHOCIDE®; Immunomedics), including epratuzumab Y-90 (Juweid et al. Cancer Res 55(23 Suppl):5899s-5907s (1995)), Abiogen's CD22 antibody (Abiogen, Italy), CMC 544 (Wyeth/Celltech), combotox (UT Soutwestern), BL22 (NIH), and LympoScan Tc99 (Immunomedics); EpCAM antibody such as 17-1A (PANOREX®); αvβ3 antibody (e.g. VITAXIN®; Medimmune); CD37 antibody such as TRU 016 (Trubion); IL-21 antibody (Zymogenetics/Novo Nordisk); anti-B cell antibody (Impheron); B cell targeting MAb (Immunogen/Aventis); 1D09C3 (Morphosys/GPC); LymphoRad 131 (HGS); Lym-1 antibody Y-90 (USC); LIF 226 (Enhanced Lifesci.); BAFF antibody (e.g., WO 03/33658); BAFF receptor antibody (e.g., WO 02/24909); BR3 antibody; Blys antibody such as belimumab; LYMPHOSCD22 -B™; anti-Lym-1 Oncolym (USC/Peregrine); ISF 154 (UCSD/Roche/Tragen); gomiliximа (Idec 152; Biogen Idec); IL-6 receptor antibody such as atlizumab (ACTEMRA™; Chugai/Roche); IL-15 antibody such as HuMax-Il-15 (Genmab/Amgen); chemokine receptor antibody, such as a CCR2 antibody (e.g. MLN1202; Millienеum); anti-complement antibody, such as C5 antibody (e.g. eculizumab, 5G1.1;

Alexion); oral formulation of human immunoglobulin (e.g. IgPO; Protein Therapeutics); IL-12 antibody such as ABT-874 (CAT/Abbott); Teneliximab (BMS-224818); B cell vaccine; DN-BAFF (Xencor); CRx-119 (CombinatoRx); Amgen's BAFF antagonist; Pentostatin (Pfizer); IC-485 (ICOS); chemokine antagonist such as T-487 (Tularik) or Reticulose (AVR-118); SCO-323 (SCIOS); integrin antagonist 683699, Tanabe, NGD-2001-1 (Neurogen); SCIO-469 (SCIOS); BIRB-796 (Boehringer Ingelheim); VX702, VX850 (Vertex); Leukotriene B-4 antagonist (such as amelubunt, BIIL-284; BI); microtubule modulator (Paxceed; Angiotech); protease inhibitor (MBS561392; BMS); AGIX-4207 (Atherogenics); ISIS-104838 (ISIS/Elan); MFG-IRAP (Univ. Pitt.); IL-1 Trap (RGN-303; Regeneron/Novartis); oprelvekin (Wyeth); everolimus (Certican; Novartis); Amevive (Biogen Idec); ORG-39141 (Organon); FK-506 (Fujisawa); and IL-2 antagonist (tacrolimus; Fujisawa).

A detailed description of exemplary anti-CD22 antibodies is as follows:

1. Specific embodiments of anti-CD22 antibodies

In one aspect, the invention provides an antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) an HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6; (d) an HVR-L1 comprising the amino acid sequence of any one of SEQ ID NO:9, 10, 19, 20, 21, 22, 23; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (f) an HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:14.

In one aspect, the invention provides an anti-CD22 antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) an HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6.

In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6 and an HVR-H1 comprising an amino acid sequence selected from SEQ ID NO:2.

In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H3 comprising an amino acid sequence selected from SEQ ID NO:6 and an HVR-H2 comprising an amino acid sequence selected from SEQ ID NO:4.

In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2 and an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4.

In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

In one aspect, the invention provides an anti-CD22 antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:10; (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and (c) an HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:14. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:9. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:10. In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:19-23. In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by V (an N28V amino acid change, which generates SEQ ID NO:10). In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by A (an N28A amino acid change, which generates SEQ ID NO:19). In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by Q (an N28Q amino acid change, which generates SEQ ID NO:20). In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by S (an N28S amino acid change,

which generates SEQ ID NO:21). In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by D (an N28D amino acid change, which generates SEQ ID NO:22). In one aspect, the HVR-L1 comprises the amino acid sequence of SEQ ID NO:9 wherein N28 is replaced by I (an N28I amino acid change, which generates SEQ ID NO:23). In one aspect, the invention provides an anti-CD22 antibody comprising an HVR-L1 comprising the amino acid sequence of any one of SEQ ID NO:9, 10, 19, 20, 21, 22, 23. In one aspect, the HVR-L1 is any one of SEQ ID NO:9, 10, 19, 20, 21, 22, or 23 and the amino acid at position N30 (asparagine at position 30) is replaced by A (an N30A amino acid change). In one aspect, the HVR-L1 is any one of SEQ ID NO:9, 10, 19, 20, 21, 22, or 23 and the amino acid at position N30 (asparagine at position 30) is replaced by Q (an N30Q amino acid change).

In one aspect, the invention provides an anti-CD22 antibody comprising (a) an HVR-H3 comprising an amino acid sequence of SEQ ID NO:6 and (b) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14. In some embodiments, the CD22 antibody further comprises (a) an HVR-H1 comprising SEQ ID NO:2 and an HVR-H2 comprising SEQ ID NO:4.

In one aspect, the invention provides an anti-CD22 antibody comprising (a) an HVR-H3 comprising an amino acid sequence of SEQ ID NO:6 and (b) an HVR-L2 comprising an amino acid sequence of SEQ ID NO:12. In some embodiments, the CD22 antibody further comprises (a) an HVR-H1 comprising SEQ ID NO:2 and an HVR-H2 comprising SEQ ID NO:4.

In one aspect, the invention provides an anti-CD22 antibody comprising (a) an HVR-H3 comprising an amino acid sequence of SEQ ID NO:6 and (b) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NO:9, 10, 19, 20, 21, 22, and 23. In some embodiments, the CD22 antibody further comprises (a) an HVR-H1 comprising SEQ ID NO:2 and an HVR-H2 comprising SEQ ID NO:4. In some embodiments, the amino acid sequence of SEQ ID NO:9, 10, 19, 20, 21, 22, or 23 comprises an N30A or N30Q amino acid change. In some embodiments, the CD22 antibody further comprises HVR-L2 comprising the amino acid sequence of SEQ ID NO:12. In some embodiments, the CD22 antibody further comprises HVR-L3 comprising the amion acid sequence of SEQ ID NO:14.

In one aspect, the invention provides an anti-CD22 antibody comprising (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6; (d) an HVR-L1 comprising the amino acid sequence selected from SEQ ID NO:9, 10, 19, 20, 21, 22, 23; (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and an HVR-L3 comprising the amion acid sequence of SEQ ID NO:14. In some embodiments, the invention further provides that the amino acid sequence SEQ ID NO:9, 10, 19, 20, 21, 22, or 23 selected as HVR-L1 is modified by an N30A or an N30Q amino acid change.

In one aspect, the invention provides an anti-CD22 antibody comprising a heavy chain variable domain comprising SEQ ID NO:16 (see Figure 2A, h10F4v1). In one aspect, the invention provides an anti-CD22 antibody comprising a light chain variable domain comprising SEQ ID NO:17 (see Figure 2B, h10F4v1). In one aspect, the invention provides an anti-CD22 antibody comprising a light chain variable domain comprising SEQ ID NO:18 (see Figure 2B, h10F4v3).

In one aspect, the invention provides an anti-CD22 antibody comprising a heavy chain comprising SEQ ID NO:34 (see Figure 2A, m10F4). In one aspect, the invention provides an anti-CD22 antibody comprising a light chain comprising SEQ ID NO:35 (see Figure 2B, m10F4).

In one aspect, the invention provides an anti-CD22 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the antibody 10F4.4.1 produced by the hybridoma deposited with the ATCC and having accession number PTA-7621.

In one aspect, the invention provides an anti-CD22 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the antibody 5E8.1.8 produced by the hybridoma deposited with the ATCC and having accession number PTA-7620.

An anti-CD22 antibody may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind CD22. For example, in some embodiments, anti-CD22 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 71, 73 and/or 78.

In one embodiments of these antibodies, position 71 is A, position 73 is T, and/or position 78 is A. In one embodiment, these antibodies comprise a heavy chain variable domain framework sequence of huMAb4D5-8, e.g., SEQ ID NO:1, 3, 5, 7 (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 □I 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:8, 1, 13, 15 (FR-L1, FR-L2, FR-L3, FR-L4, respectively).

In one embodiment, an anti-CD22 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:1, 3, 5, and 7, respectively; the HVR H1 comprises the amino acid sequence of SEQ ID NO:2; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:4; and the HVR-H3 comprises an amino acid sequence selected from SEQ ID NO:6. In one embodiment, an anti-CD22 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 NO:8, 11, 13, and 15, respectively; the HVR-L1 comprises the amino acid sequence selected from SEQ ID NO:9, 10, 19, 20, 21, 22, and 23, wherein any one of SEQ ID NOS:9-10 or 19-23 may comprise a N30A or N30Q amino acid change; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:12; and the HVR-L3 comprises an amino acid sequence selected from SEQ ID NO:14. In one embodiment of these antibodies, the heavey chain variable domain comprises SEQ ID NO:16 and the light chain variable domain comprises SEQ ID NO:17 or 18.

In some embodiments, the invention provides an anti-CD22 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:16. 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 CD22. In some

embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence SEQ ID NO:16. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-CD22 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NO:16.

In some embodiments, the invention provides an anti-CD22 antibody comprising a heavy chain variable domain as depicted in below.

1 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 113 (SEQ ID NO:16) (HVR residues are underlined).

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

HVR-H1 (Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn, SEQ ID NO:2)

HVR-H2 (Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys Phe Lys Gly, SEQ ID NO:4)

HVR-H3 (Asp Gly Ser Ser Trp Asp Try Tyr Phe Asp Tyr, SEQ ID NO:6)

FR-H1 (Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser, SEQ ID NO:1)

FR-H2 (Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val, SEQ ID NO:3)

FR-H3 (Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg, SEQ ID NO:5)

FR-H4 (Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser, SEQ ID NO:7)

In some embodiments, the invention provides an anti-CD22 antibody comprising a light chain variable domain as depicted in below.

1 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro

Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 108 (SEQ ID NO:17) (HVR residues are underlined and position N28 is in bold type)

Or

1 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 108 (SEQ ID NO:18) (HVR residues are underlined and position N28V is in bold type).

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

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu Glu, SEQ ID NO:9)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu Glu, SEQ ID NO:10)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ala Gly Asn Thr Phe Leu Glu, SEQ ID NO:19)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Gln Gly Asn Thr Phe Leu Glu, SEQ ID NO:20)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Asn Thr Phe Leu Glu, SEQ ID NO:21)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Asp Gly Asn Thr Phe Leu Glu, SEQ ID NO:22)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Asn Thr Phe Leu Glu, SEQ ID NO:23)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Ala Thr Phe Leu Glu, SEQ ID NO:32)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Gln Thr Phe Leu Glu, SEQ ID NO:33)

HVR-L2 (Lys Val Ser Asn Arg Phe Ser, SEQ ID NO:12)

HVR-L3 (Phe Gln Gly Ser Gln Phe Pro Tyr Thr, SEQ ID NO:14).

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

FR-L1 (Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys, SEQ ID NO:8);

FR-L2 (Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr, SEQ ID NO:11);

FR-L3 (Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys, SEQ ID NO:13)

FR-L4 (Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg, SEQ ID NO:15).

In one aspect, the invention provides an anti-CD22 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 selected from SEQ ID NO:17 or 18. 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 CD22. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a sequence selected from SEQ ID NO:17 or 18. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-CD22 antibody comprises a light chain variable domain comprising an amino acid sequence selected from SEQ ID NO:17 or 18.

In one aspect, the invention provides an anti-CD22 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:16; 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 selected from SEQ ID NO:17 or 18. 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 CD22. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in the reference sequence. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-CD22 antibody comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:16 and a light chain variable domain comprising an amino acid sequence selected from SEQ ID NO:18.

In one aspect, the invention provides an anti-CD22 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Figure 2A and/or (b) one, two, or three VL HVRs selected from those shown in Figure 2B. In one aspect, the invention provides an anti-CD22 antibody comprising a heavy chain variable domain selected from those shown in Figure 2A and a light chain variable domain selected from those shown in Figure 2B.

In one aspect, the anti-CD22 antibody of the invention comprises 1, 2, 3, 4, 5, or 6 of the hypervariable regions of the 5E8.1.8 antibody produced by the hybridoma deposited with the ATCC and having accession no. PTA-7620.

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 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 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-CD22 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-CD22 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-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 CD22 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD22. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD22. These antibodies possess a CD22-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 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 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 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 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, 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).

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

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 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 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.

Certain Methods of Making Antibodies

1. Certain Hybridoma-Based Methods

The anti-CD22 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 CD22 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of CD22 and an adjuvant. CD22 may be prepared using methods well-known in the art, some of which are further described herein. For example, CD22 may be produced recombinantly. In one embodiment, animals are immunized with a derivative of CD22 that contains an extracellular portion of CD22 fused to the Fc portion of an immunoglobulin heavy chain. In one embodiment, animals are immunized with an CD22-IgG1 fusion protein. In one embodiment, animals are immunized with immunogenic derivatives of CD22 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- CD22 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 CD22. 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-CD22 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-CD22 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-CD22 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).

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-CD22 clones is desired, the subject is immunized with CD22 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-CD22 clones is obtained by generating an anti-CD22

antibody response in transgenic mice carrying a functional human immunoglobulin gene array (and lacking a functional endogenous antibody production system) such that CD22 immunization gives rise to B cells producing human antibodies against CD22. The generation of human antibody-producing transgenic mice is described below.

Additional enrichment for anti-CD22 reactive cell populations can be obtained by using a suitable screening procedure to isolate B cells expressing CD22-specific membrane bound antibody, e.g., by cell separation using CD22 affinity chromatography or adsorption of cells to fluorochrome-labeled CD22 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 CD22 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 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 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, CD22 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 CD22 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 CD22 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 CD22. 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 CD22, rare high affinity phage could be competed out. To retain all higher affinity mutants, phages can be incubated with excess biotinylated CD22, but with the biotinylated CD22 at a concentration of lower molarity than the target molar affinity constant for CD22. 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-CD22 clones may be selected based on activity. In certain embodiments, the invention provides anti-CD22 antibodies that bind to living cells that naturally express CD22. In one embodiment, the invention provides anti-CD22 antibodies that block the binding between a CD22 ligand and CD22, but do not block the binding between a CD22 ligand and a second protein. Fv clones corresponding to such anti-CD22 antibodies can be selected by (1) isolating anti-CD22 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 CD22 and a second protein against which blocking and non-blocking activity, respectively, is desired; (3) adsorbing the anti-CD22 phage clones to immobilized CD22; (4) using an excess of the second protein to elute any undesired clones that recognize CD22-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-CD22 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 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.

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, lpp, 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 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.

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.

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).

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 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 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 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.

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

The invention also provides immunoconjugates (interchangeably referred to as "antibody-drug conjugates," or "ADCs") comprising any of the anti-CD22 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-CD22 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-CD22 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 – Antibody Drug Conjugates

An immunoconjugate (or "antibody-drug conjugate" ("ADC")) of the invention may be of Formula I, below, wherein an anti-CD22 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-CD22 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.

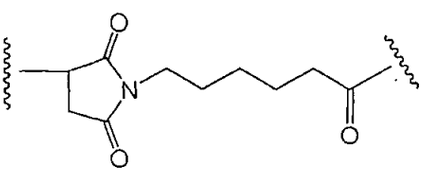
Exemplary Linkers

Exemplary linkers and drug moieties are disclosed herein. A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl

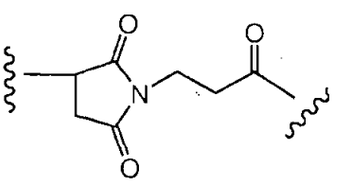
("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC"), and N-Succinimidyl (4-iodo-acetyl) 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, 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.

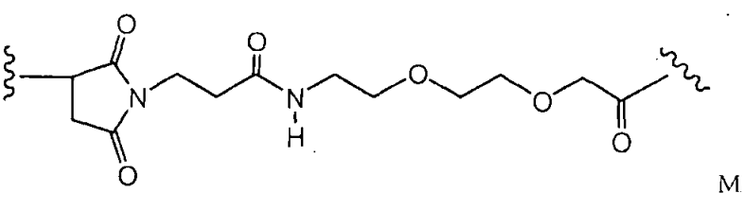
In some embodiments, a linker component may comprise a "stretcher unit" that links an antibody to another linker component or to a drug moiety. Exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody):



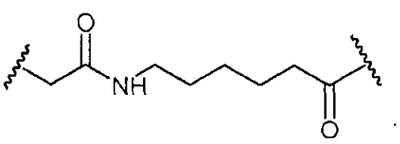
MC



MP



MPEG



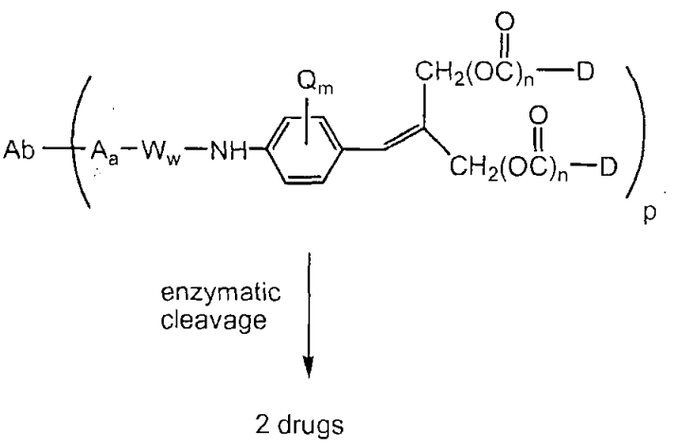
In some embodiments, a linker component may comprise an amino acid unit. In one such embodiment, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes. 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-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.

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.



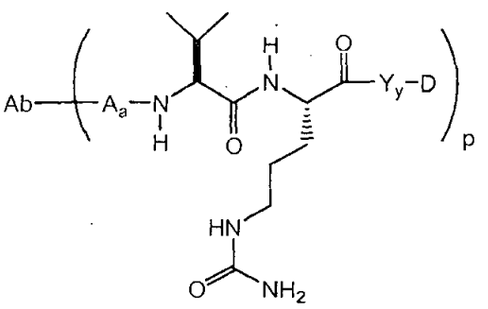
wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

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

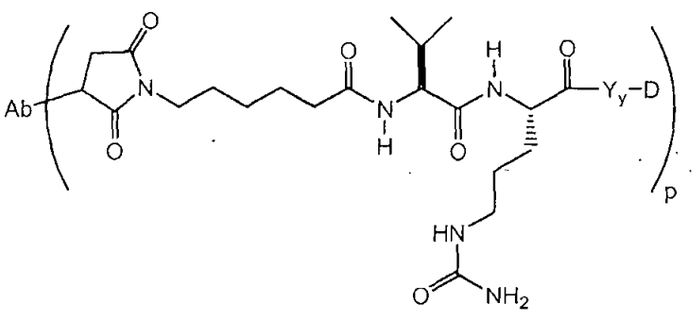
$$Ab\text{-}([Aa\text{-}Ww\text{-}Yy]\text{-}D)_p \qquad \text{Formula II}$$

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.

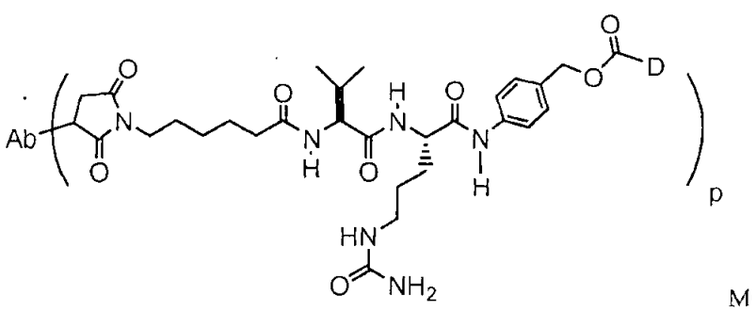
Exemplary linker components and combinations thereof are shown below in the context of ADCs of Formula II:



Val-Cit or VC



MC-val-cit



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.

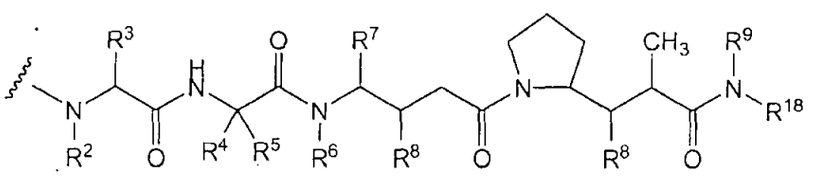
Exemplary embodiments of maytansinoid drug moieities include: DM1; DM3; and DM4, as disclosed herein.

Auristatins and dolastatins

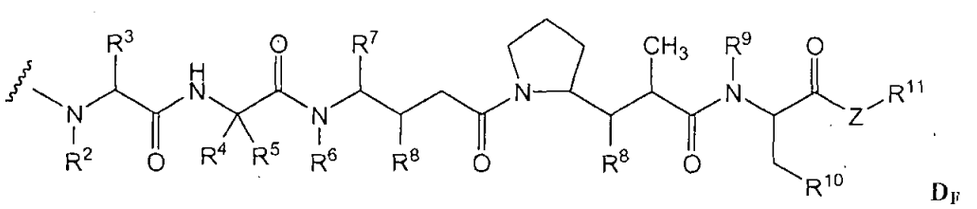
In some embodiments, an immunoconjugate comprises an antibody of the invention conjugated to dolastatin or a dolastatin peptidic analog or derivative, e.g., an auristatin (US Pat. Nos. 5635483; 5780588). Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, 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;

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.

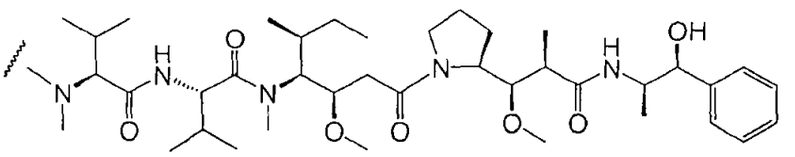
In an exemplary embodiment, R^{10} is -phenyl.

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 $-(CH_2)_n-COOH$.

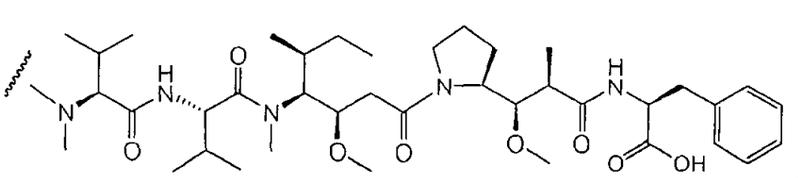
In another embodiment, when Z is -NH, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

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:



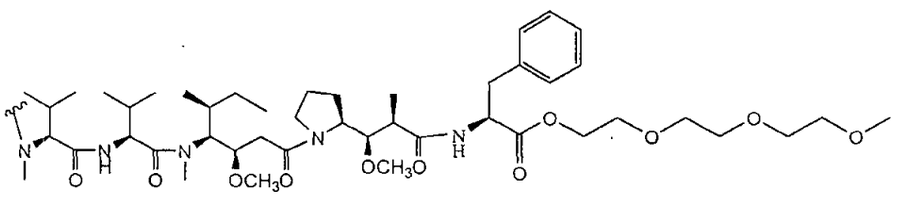
MMAE

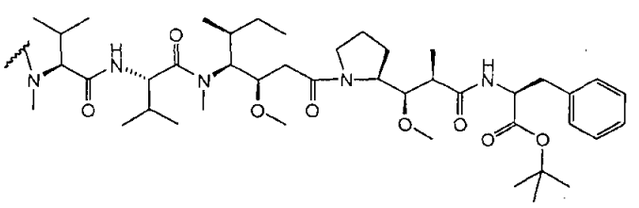
An exemplary auristatin embodiment of formula D_F is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate (*see* US 2005/0238649 and Doronina et al. (2006) *Bioconjugate Chem.* 17:114-124):

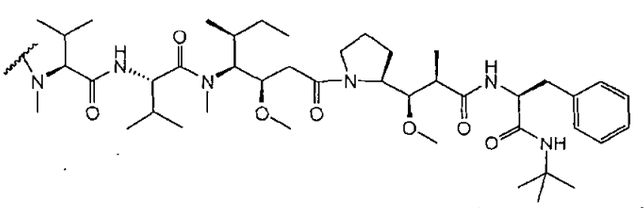


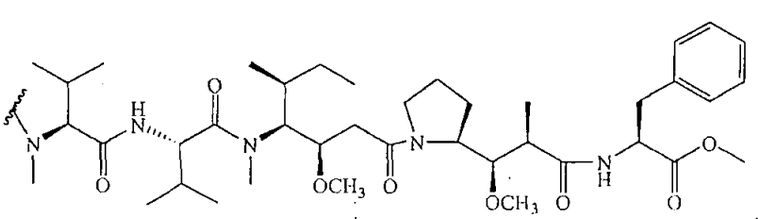
MMAF

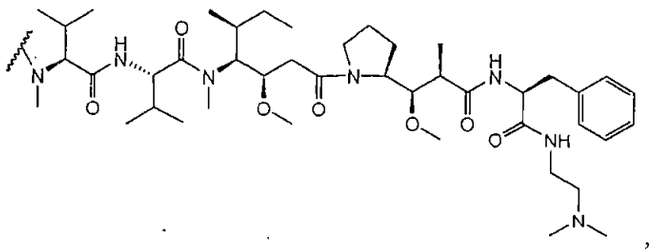
Other drug moieties include the following MMAF derivatives, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



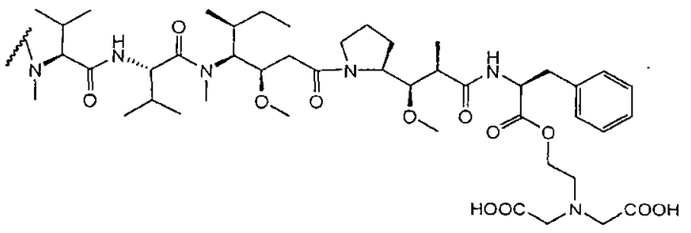




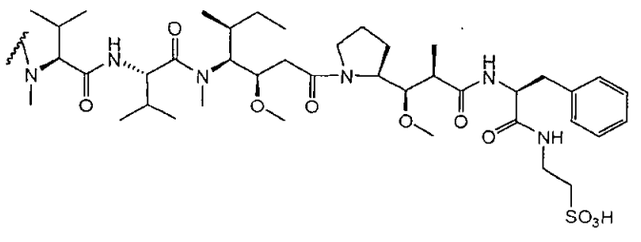




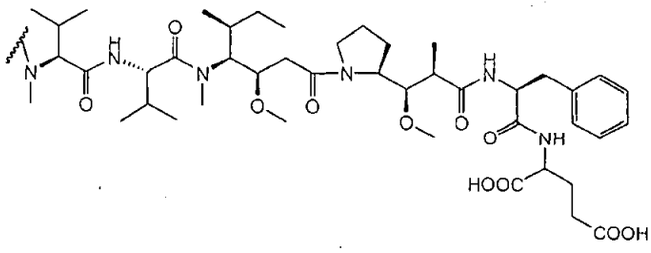
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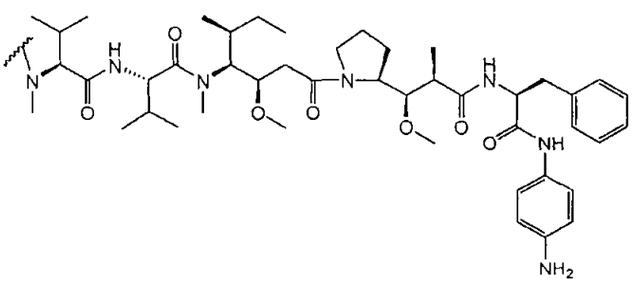
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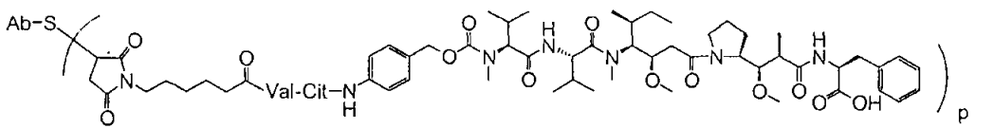


, and

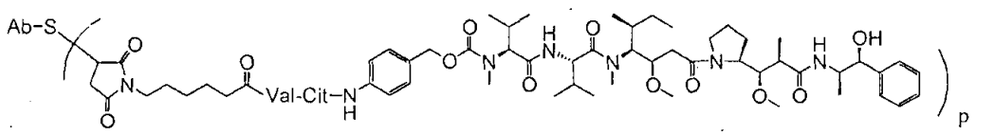


In one aspect, hydrophilic groups including but not limited to, triethylene glycol esters (TEG), as shown above, can be attached to the drug moiety at R^{11}. Without being bound by any particular theory, the hydrophilic groups assist in the internalization and non-agglomeration of the drug moiety.

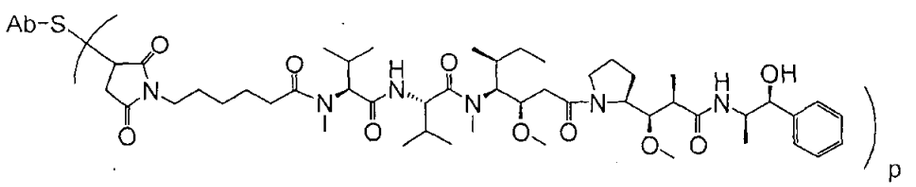
Exemplary embodiments of ADCs of Formula I comprising an auristatin/dolastatin or derivative thereof are described in US 2005-0238649 A1 and Doronina et al. (2006) Bioconjugate Chem. 17:114-124, which is expressly incorporated herein by reference. Exemplary embodiments of ADCs of Formula I comprising 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:



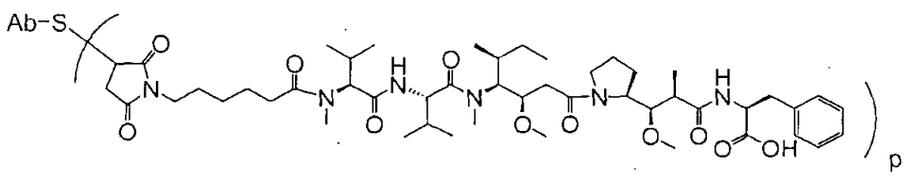
Ab-MC-vc-PAB-MMAF



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 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 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. Only the most reactive lysine groups may react with an amine-reactive linker reagent. 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.

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. Alternatively, sulfhydryl 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.

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).

2. *Exemplary Immunoconjugates – Thio-Antibody Drug Conjugates*

Preparation of cysteine engineered anti-CD22 antibodies

DNA encoding an amino acid sequence variant of the cysteine engineered anti-CD22 antibodies and parent anti-CD22 antibodies of the invention is prepared by a variety of methods which include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants), preparation by site-directed (or oligonucleotide-mediated) mutagenesis (Carter (1985) et al Nucleic Acids Res. 13:4431-4443; Ho et al (1989) Gene (Amst.).77:51-59; Kunkel et al (1987) Proc. Natl. Acad. Sci.

USA 82:488; Liu et al (1998) J. Biol. Chem. 273:20252-20260), PCR mutagenesis (Higuchi, (1990) in PCR Protocols, pp.177-183, Academic Press; Ito et al (1991) Gene 102:67-70; Bernhard et al (1994) Bioconjugate Chem. 5:126-132; and Vallette et al (1989) Nuc. Acids Res. 17:723-733), and cassette mutagenesis (Wells et al (1985) Gene 34:315-323) of an earlier prepared DNA encoding the polypeptide. Mutagenesis protocols, kits, and reagents are commercially available, e.g. QuikChange® Multi Site-Direct Mutagenesis Kit (Stratagene, La Jolla, CA). Single mutations are also generated by oligonucleotide directed mutagenesis using double stranded plasmid DNA as template by PCR based mutagenesis (Sambrook and Russel, (2001) Molecular Cloning: A Laboratory Manual, 3rd edition; Zoller et al (1983) Methods Enzymol. 100:468-500; Zoller, M.J. and Smith, M. (1982) Nucl. Acids Res. 10:6487-6500). Variants of recombinant antibodies may be constructed also by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. Mutagenic primers encode the cysteine codon replacement(s). Standard mutagenesis techniques can be employed to generate DNA encoding such mutant cysteine engineered antibodies (Sambrook et al Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Ausubel et al Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York, N.Y., 1993).

Phage display technology (McCafferty et al (1990) Nature 348:552-553) can be used to produce anti-CD22 human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell (Johnson et al (1993) Current Opinion in Structural Biology 3:564-571; Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol. 222:581-597; Griffith et al (1993) EMBO J. 12:725-734; US 5565332; US 5573905; US 5567610; US 5229275).

Anti-CD22 antibodies may be chemically synthesized using known oligopeptide synthesis methodology or may be prepared and purified using recombinant technology. The

appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (Stewart et al., Solid-Phase Peptide Synthesis, (1969)W.H. Freeman Co., San Francisco, CA; Merrifield, (1963) J. Am. Chem. Soc., 85:2149-2154). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated solid phase synthesis may be accomplished, for instance, employing t-BOC or Fmoc protected amino acids and using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the anti-CD22 antibody or CD22 polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired anti-CD22 antibody or CD22 polypeptide.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (Morimoto et al (1992) Journal of Biochemical and Biophysical Methods 24:107-117; and Brennan et al (1985) Science, 229:81), or produced directly by recombinant host cells. Fab, Fv and ScFv anti-CD22 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 herein. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al (1992) Bio/Technology 10:163-167), or isolated directly from recombinant host cell culture. The anti-CD22 antibody may be a (scFv) single chain Fv fragment (WO 93/16185; US 5571894; US. 5587458). The anti-CD22 antibody fragment may also be a "linear antibody" (US 5641870). Such linear antibody fragments may be monospecific or bispecific.

The description below relates primarily to production of anti-CD22 antibodies by culturing cells transformed or transfected with a vector containing anti-CD22 antibody-encoding nucleic acid. DNA encoding anti-CD22 antibodies may be obtained from a cDNA library prepared from tissue believed to possess the anti-CD22 antibody mRNA and to express it at a detectable level. Accordingly, human anti-CD22 antibody or CD22 polypeptide DNA can be conveniently obtained from a cDNA library prepared from human tissue. The anti-CD22 antibody-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

The design, selection, and preparation methods of the invention enable cysteine engineered anti-CD22 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) 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.

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-CD22 antibody is prepared by a process comprising:

(a) replacing one or more amino acid residues of a parent anti-CD22 antibody by cysteine; and

(b) determining the thiol reactivity of the cysteine engineered anti-CD22 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.

Another embodiment of the invention provides a method of preparing (making) a cysteine engineered anti-CD22 antibody, comprising:

(a) introducing one or more cysteine amino acids into a parent anti-CD22 antibody in order to generate the cysteine engineered anti-CD22 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.

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.

Cysteine engineering of anti-CD22 10F4 IgG variants

Cysteine was introduced at the heavy chain 118 (EU numbering) (equivalent to heavy chain position 121, sequential numbering) site into the full-length, chimeric parent monoclonal anti-CD22 antibodies by the cysteine engineering methods described herein.

The parent antibody, "std Anti-CD22 Hu 10F4v3 Fc" (Heavy Chain sequence: SEQ ID NO:88, Light Chain sequence: SEQ ID NO:87, Figure 5B) was cysteine engineered to give "A118C thio hu anti-CD22 10F4v3" (Heavy Chain sequence: SEQ ID NO:92, Light Chain sequence: SEQ ID NO:91, Figure 17), "S400C thio hu anti-CD22 10F4v3" (Heavy Chain sequence: SEQ ID NO:93, Light Chain sequence: SEQ ID NO:91, Figure 17), or "V205C thio anti-CD22 10F4v3" (Heavy Chain sequence: SEQ ID NO:88, Light Chain sequence: SEQ ID NO:91, Figures 5B and 17).

These cysteine engineered monoclonal antibodies were expressed in CHO (Chinese Hamster Ovary) cells by transient fermentation in media containing 1 mM cysteine.

Labelled cysteine engineered anti-CD22 antibodies

Cysteine engineered anti-CD22 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-CD22 antibodies

Cysteine engineered anti-CD22 antibodies, and conjugates thereof may find use as therapeutic and/or diagnostic agents. The present invention further provides methods of preventing, managing, treating or ameliorating one or more symptoms associated with a B-cell 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., 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. The present invention still further provides methods for diagnosing a CD22 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 B cell-associated CD22 polypeptides.

Another embodiment of the present invention is directed to the use of a cysteine engineered anti-CD22 antibody for the preparation of a medicament useful in the treatment of a condition which is responsive to a B cell related disorder.

Cysteine Engineered Antibody Drug Conjugates (Thio-antibody Drug Conjugates)

Another aspect of the invention is an antibody-drug conjugate compound comprising a cysteine engineered anti-CD22 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:

$$Ab-(L-D)_p \qquad I$$

where p is 1, 2, 3, or 4; and wherein the cysteine engineered antibody is prepared by a process comprising replacing one or more amino acid residues of a parent anti-CD22 antibody by one or more free cysteine amino acids.

Figure 10 shows embodiments of cysteine engineered anti-CD22 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).

Potential advantages of cysteine engineered anti-CD22 antibody drug conjugates include improved safety (larger therapeutic index), improved PK parameters, the antibody inter-chain disulfide bonds are retained which may stabilize the conjugate and retain its active binding conformation, the sites of drug conjugation are defined, and the preparation of of cysteine engineered antibody drug conjugates from conjugation of cysteine engineered antibodies to drug-linker reagents results in a more homogeneous product.

Linkers

"Linker", "Linker Unit", or "link" means a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, a linker is specified as L. A "Linker" (L) is a bifunctional or multifunctional moiety which can be used to link one or more Drug moieties (D) and an antibody unit (Ab) to form antibody-drug conjugates (ADC) of Formula I. Antibody-drug conjugates (ADC) can be conveniently prepared using a Linker having reactive functionality for binding to the Drug and to the Antibody. A cysteine thiol of a cysteine engineered antibody (Ab) can form a bond with an electrophilic functional group of a linker reagent, a drug moiety or drug-linker intermediate.

In one aspect, a Linker has a reactive site which has an electrophilic group that is reactive to a nucleophilic cysteine present on an antibody. The cysteine thiol of the antibody is reactive with an electrophilic group on a Linker and forms a covalent bond to a Linker. Useful electrophilic groups include, but are not limited to, maleimide and haloacetamide groups.

Linkers include a divalent radical such as an alkyldiyl, an arylene, a heteroarylene, 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.

Cysteine engineered antibodies react with linker reagents or drug-linker intermediates, with electrophilic functional groups such as maleimide or α-halo carbonyl,

according to the conjugation method at page 766 of Klussman, et al (2004), Bioconjugate Chemistry 15(4):765-773, and according to the protocol of Example x.

The linker may be composed of one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe" or "af"), 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"), ethyleneoxy $-CH_2CH_2O-$ as one or more repeating units ("EO" or "PEO"). Additional linker components are known in the art and some are described herein.

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 functional group that can form a bond with a functional group of a Stretcher. Useful functional groups that can be present on an antibody, either naturally or via chemical manipulation include, but are not limited to,

sulfhydryl (-SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl. In one aspect, the antibody functional groups are sulfhydryl or amino. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of an antibody. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of an antibody using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent. In one embodiment, 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.

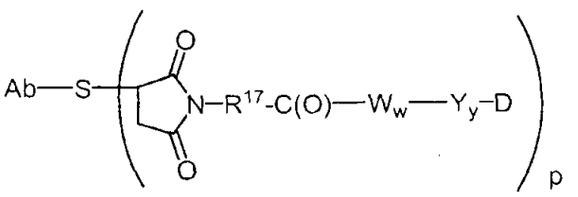
Heterocyclyl groups include a ring system in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 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 [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.

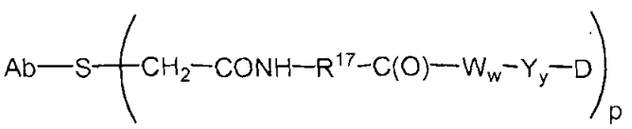
Examples of heterocycles include by way of example and not limitation pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, 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.

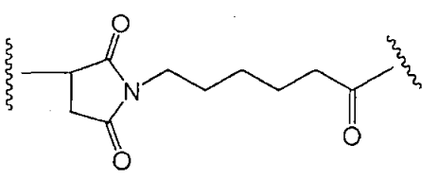


II



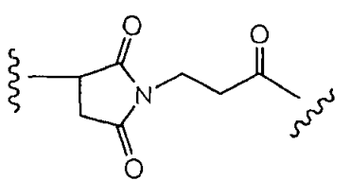
III

An illustrative Formula II Stretcher unit is derived from maleimido-caproyl (MC) wherein R^{17} is $-(CH_2)_5-$:



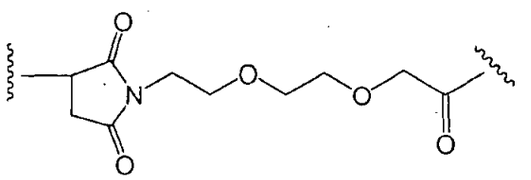
MC

An illustrative Stretcher unit of Formula II, and is derived from maleimido-propanoyl (MP) wherein R^{17} is $-(CH_2)_2-$:

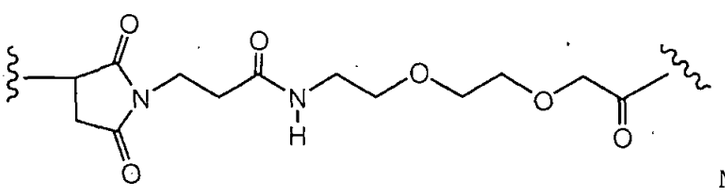


MP

Another illustrative Stretcher unit of Formula II wherein R^{17} is $-(CH_2CH_2O)_r-CH_2-$ and r is 2:

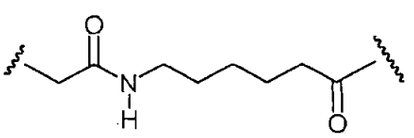


Another illustrative Stretcher unit of Formula II wherein R^{17} is $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$ where R^b is H and each r is 2:

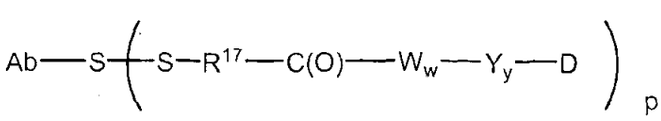


MPEG

An illustrative Stretcher unit of Formula III wherein R^{17} is $-(CH_2)_5-$:

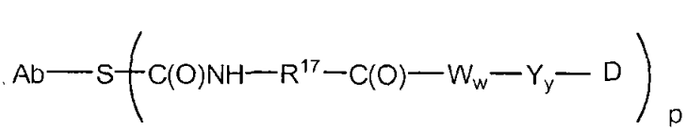


In another embodiment, the Stretcher unit is linked to the cysteine engineered anti-CD22 antibody via a disulfide bond between the engineered cystein sulfur atom of the antibody and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted by Formula IV, wherein R^{17}, Ab-, -W-, -Y-, -D, w and y are as defined above.

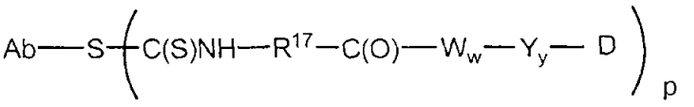


IV

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



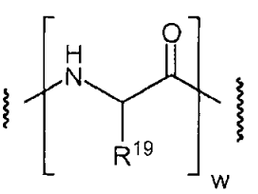
Vb

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

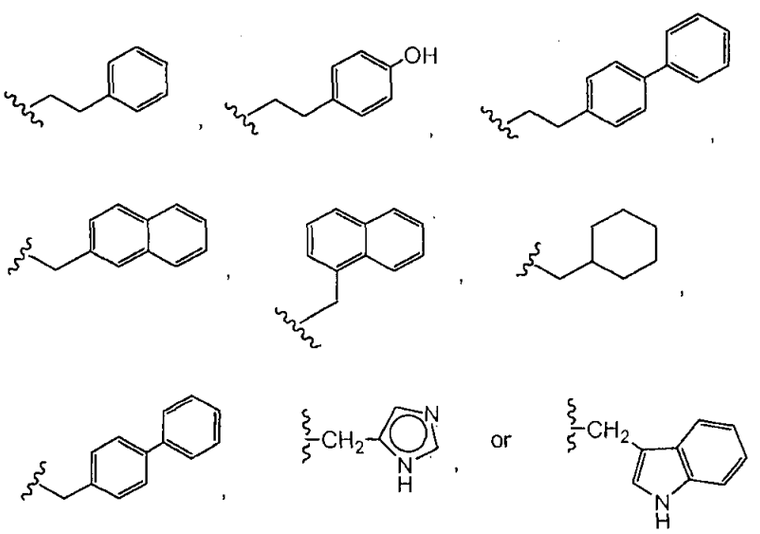
The linker may comprise amino acid residues. The Amino Acid unit ($-W_w-$), when present, links the antibody (Ab) to the drug moiety (D) of the cysteine engineered antibody-drug conjugate (ADC) of the invention.

$-W_w-$ 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 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.

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

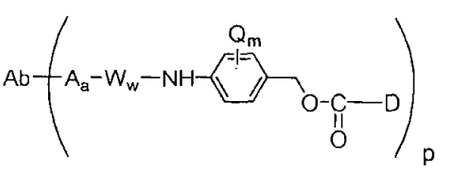
The Spacer unit (–Y_y–), when present (y = 1 or 2), links an Amino Acid unit (–W_w–) to the drug moiety (D) when an Amino Acid unit is present (w = 1-12). Alternately, the Spacer unit links the Stretcher unit to the Drug moiety when the Amino Acid unit is absent. The Spacer unit also links the drug moiety 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.

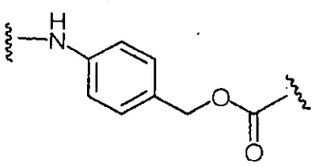
Alternatively, an ADC containing a self-immolative Spacer unit can release -D. In one embodiment, -Y- is a PAB group that is linked to -W_w- via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group, where the ADC has the exemplary structure:



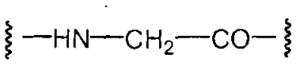
wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to 4.

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.

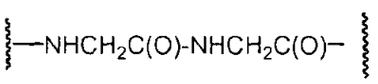
Exemplary Spacer units (-Y_y-) are represented by Formulas X-XII:



X



XI



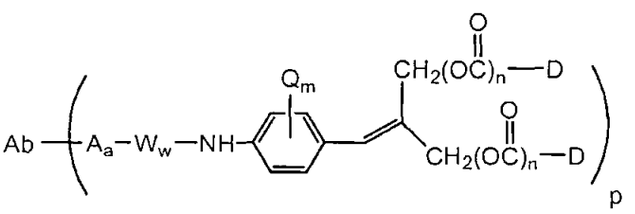
XII

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

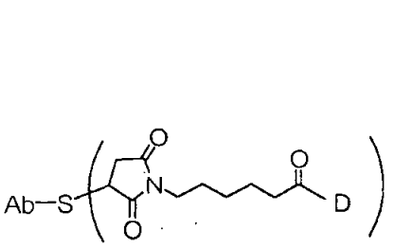
the ADC. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker. Exemplary embodiments of branched, dendritic linkers include 2,6-bis(hydroxymethyl)-p-cresol and 2,4,6-tris(hydroxymethyl)-phenol 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:

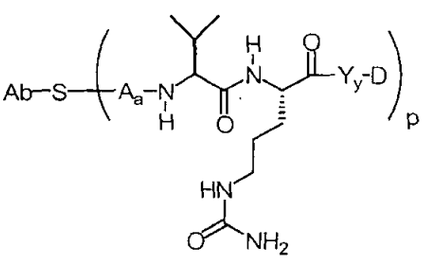


comprising a 2-(4-aminobenzylidene)propane-1,3-diol dendrimer unit (WO 2004/043493; de Groot et al (2003) Angew. Chem. Int. Ed. 42:4490-4494), wherein Q is -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges ranging from 1 to 4.

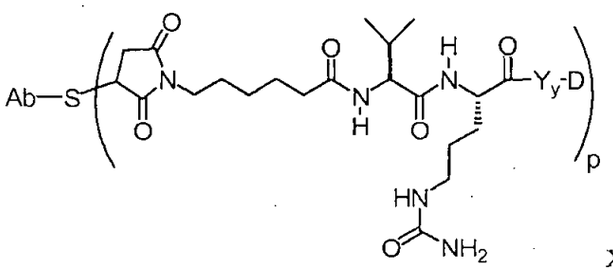
Exemplary embodiments of the Formula I antibody-drug conjugate compounds include XIIIa (MC), XIIIb (val-cit), XIIIc (MC-val-cit), and XIIId (MC-val-cit-PAB):



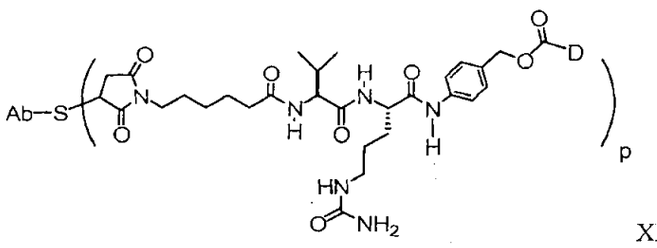
XIIIa



XIIIb

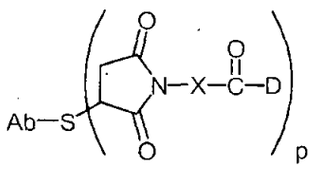


XIIIc

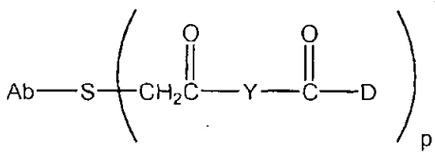


XIIId

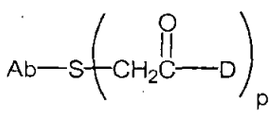
Other exemplary embodiments of the Formula Ia antibody-drug conjugate compounds include XIVa-e:



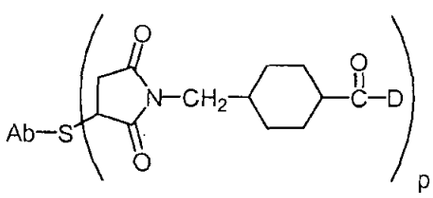
XIVa



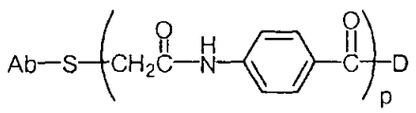
XIVb



XIVc

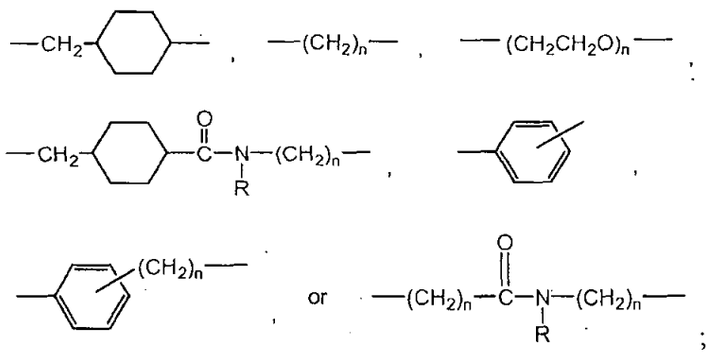


XIVd

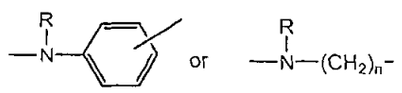


XIVe

where X is:



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.

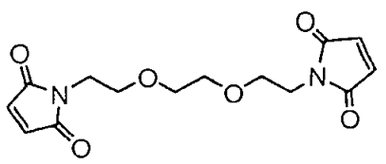
In another embodiment, the Linker may be substituted with groups which modulated solubility or reactivity. For example, a charged substituent such as sulfonate ($-SO_3^-$) or ammonium, may increase water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody 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.

Linker reagents

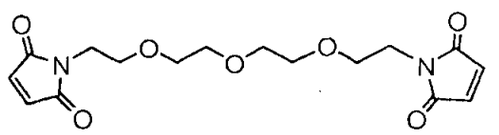
Conjugates of the antibody and auristatin may be made using a variety of bifunctional linker reagents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as 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).

The antibody drug conjugates may also be prepared with linker reagents: BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate), and including bis-maleimide reagents: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, and $BM(PEO)_4$, which are commercially available from Pierce Biotechnology, Inc., Customer Service Department, P.O. Box 117, Rockford, IL. 61105 U.S.A, U.S.A 1-800-874-3723, International +815-968-0747. Bis-maleimide reagents allow the attachment of the thiol

group of a cysteine engineered antibody to a thiol-containing drug moiety, label, or linker intermediate, in a sequential or concurrent fashion. Other functional groups besides maleimide, which are reactive with a thiol group of a cysteine engineered antibody, drug moiety, label, or linker intermediate include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.



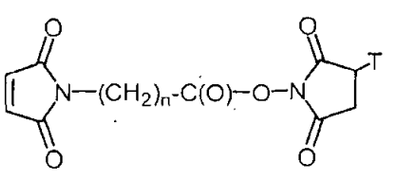
$BM(PEO)_3$



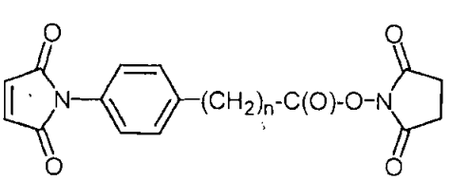
$BM(PEO)_4$

Useful linker reagents can also be obtained via other commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or synthesized in accordance with procedures described in Toki et al (2002) J. Org. Chem. 67:1866-1872; Walker, M.A. (1995) J. Org. Chem. 60:5352-5355; Frisch et al (1996) Bioconjugate Chem. 7:180-186; US 6214345; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

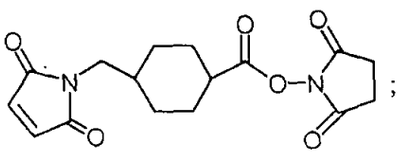
Stretchers of formula (IIIa) can be introduced into a Linker by reacting the following linker reagents with the N-terminus of an Amino Acid unit:



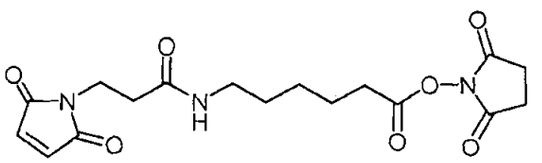
where n is an integer ranging from 1-10 and T is -H or $-SO_3Na$;



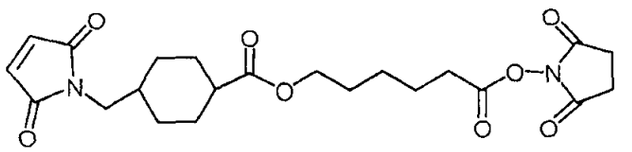
where n is an integer ranging from 0-3;



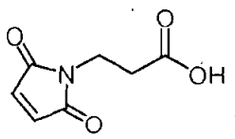
;



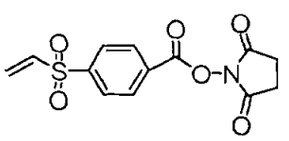
;

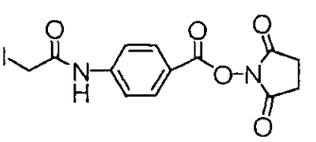


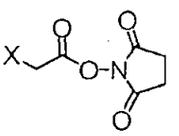
; and

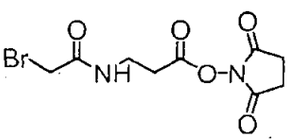


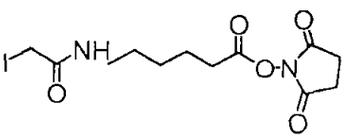
Stretcher units of can be introduced into a Linker by reacting the following bifunctional reagents with the N-terminus of an Amino Acid unit:





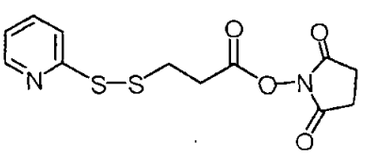


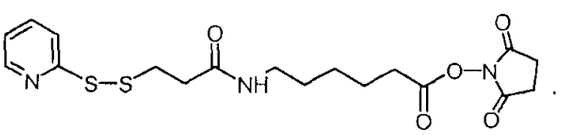


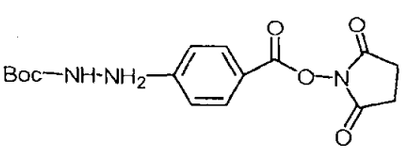


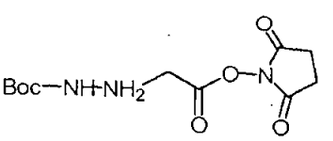
where X is Br or I.

Stretcher units of formula can also be introduced into a Linker by reacting the following bifunctional reagents with the N-terminus of an Amino Acid unit:

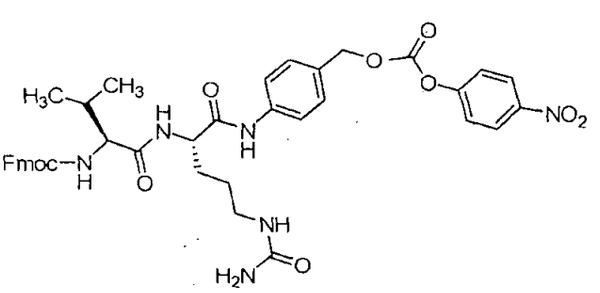




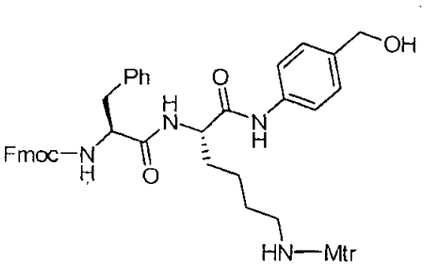




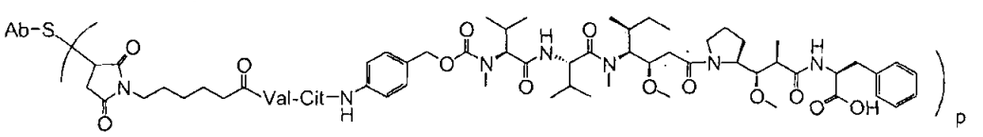
An exemplary valine-citrulline (val-cit or vc) dipeptide linker reagent having a maleimide Stretcher and a para-aminobenzylcarbamoyl (PAB) self-immolative Spacer has the structure:



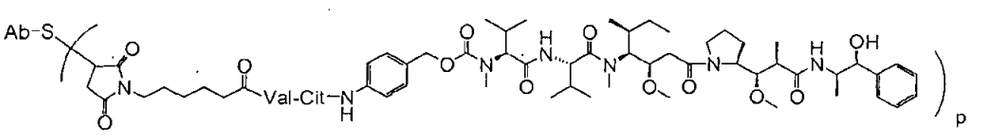
An exemplary phe-lys(Mtr, mono-4-methoxytrityl) dipeptide linker reagent having a maleimide Stretcher unit and a PAB self-immolative Spacer unit can be prepared according to Dubowchik, et al. (1997) Tetrahedron Letters, 38:5257-60, and has the structure:



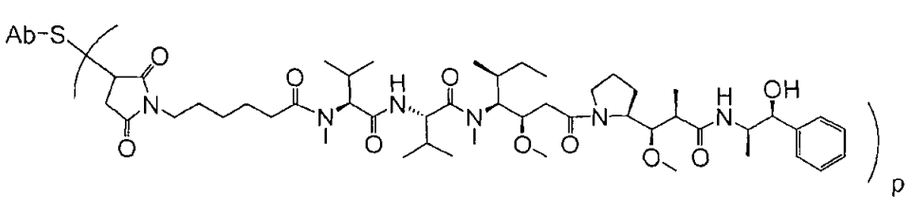
Exemplary antibody-drug conjugate compounds of the invention include:



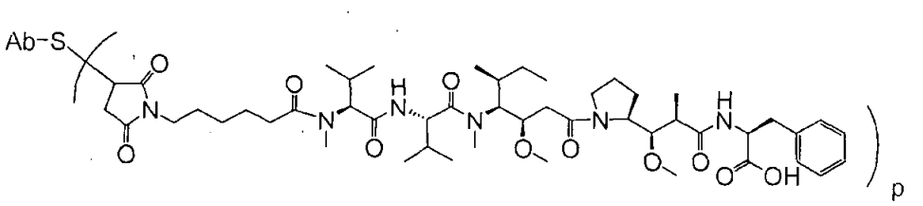
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

where Val is valine; Cit is citrulline; p is 1, 2, 3, or 4; and Ab is a cysteine engineered anti-CD22 antibody.

Preparation of cysteine engineered anti-CD22 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 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 10F4v3 anti-CD22 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 12 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.

Methods of screening

Yet another embodiment of the present invention is directed to a method of determining the presence of a CD22 polypeptide in a sample suspected of containing the CD22 polypeptide, wherein the method comprises exposing the sample to a cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, that binds to the CD22 polypeptide and determining binding of the cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, to the CD22 polypeptide in the sample, wherein the presence of such binding is indicative of the presence of the CD22 polypeptide in the sample. Optionally, the sample may contain cells (which may be cancer cells) suspected of expressing the CD22 polypeptide. The cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, employed in the method may optionally be detectably labeled, attached to a solid support, or the like.

Another embodiment of the present invention is directed to a method of diagnosing the presence of a tumor in a mammal, wherein the method comprises (a) contacting a test sample comprising tissue cells obtained from the mammal with a cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, that binds to a CD22 polypeptide and (b) detecting the formation of a complex between the cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, and the CD22 polypeptide in the test sample, wherein the formation of a complex is indicative of the presence of a tumor in the mammal. Optionally, the cysteine engineered anti-CD22 antibody, or antibody drug conjugate thereof, is detectably labeled, attached to a solid support, or the like, and/or the test sample of tissue cells is obtained from an individual suspected of having a cancerous tumor.

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. ^{14}C or ^{3}H) 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.

Pharmaceutical Formulations

Administration of Antibody-Drug Conjugates, including Thio-antibody Drug Conjugates

The antibody-drug conjugates (ADC), including thio-antibody drug conjugates (TDC), 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 these cancers, 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 alleviating an autoimmune disease, comprising administering to a patient suffering from the autoimmune disease, a therapeutically effective amount of a humanized 10F4 antibody-drug conjugate of any one of the preceding embodiments. In preferred embodiments the antibody is administered intravenously or subcutaneously. The antibody-drug conjugate is administered intravenously at a dosage in the range of about 1 $\mu g/m^2$ to about 100 mg/ m^2 per dose and in a specific embodiment, the dosage is 1 $\mu g/m^2$ to about 500 $\mu g/m^2$. 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 relief from or alleviation of symptoms of the autoimmune disease being treated. Administration may continue after relief from or alleviation of symptoms is achieved where such alleviation or relief is prolong by such continued administration.

The invention also provides a method of treating a B cell disorder comprising administering to a patient suffering from a B cell disorder, such as a B cell proliferative disorder (including without limitation lymphoma and leukemia) or an autoimmune disease, a therapeutically effective amount of a humanized 10F4 antibody of any one of the preceding embodiments, which antibody is not conjugated to a cytotoxic molecule or a detectable molecule. The anatibody 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-CD22 antibody of the invention and/or at least one immunoconjugate thereof and/or at least one anti-CD22 antibody-drug conjugate of the invention. In some embodiments, a pharmaceutical formulation comprises 1) an anti-CD22 antibody and/or an anti-CD22 antibody-drug conjugate and/or an immunoconjugate thereof, and 2) a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical formulation comprises 1) an anti-CD22 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.

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.

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-CD22 monoclonal antibody or immunoconjugate of the invetion in patients experiencing a B cell proliferative disorder including without limitation 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. 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.

Generally, the disease or disorder to be treated is a hyperproliferative disease such as a B cell proliferative disorder and/or a B cell cancer. Examples of cancer to be treated herein include, but are not limited to, B cell proliferative disorder is selected from 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.

The cancer may comprise CD22-expressing cells, such that the ADC of the present invention are able to bind to the cancer cells. To determine CD22 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, CD22 overexpression may be analyzed by IHC. Parrafin-embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a CD22 protein staining intensity criteria with respect to the degree of staining and in what prpoportion 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-ErbB2 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 a second compound having anti-cancer properties. The second 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 second 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-CD22 ADC of the invention and the second compound is an anti-CD20 antibody (either a naked antibody or an ADC). In one embodiment the second compound is an anti-CD20 antibody rituximab (Rituxan®) or 2H7 (Genentech, Inc., South San Francisco, CA). Another antibodies useful for combined immunotherapy with anti-CD22 ADCs of the invention includes without limitation, anti-VEGF (e.g, Avastin®).

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-CD20 (e.g., Rituxan®) 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 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-CD22 Antibodies and Immunoconjugates

Diagnostic methods and methods of detection

In one aspect, anti-CD22 antibodies and immunoconjugates of the invention are useful for detecting the presence of CD22 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 CD22 at higher levels relative to other tissues, for example, B cells and/or B cell associated tissues.

In one aspect, the invention provides a method of detecting the presence of CD22 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti-CD22 antibody under conditions permissive for binding of the anti-CD22 antibody to CD22, and detecting whether a complex is formed between the anti-CD22 antibody and CD22.

In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of CD22. In certain embodiments, the method comprises contacting a test cell with an anti-CD22 antibody; determining the level of expression (either quantitatively or qualitatively) of CD22 by the test cell by detecting binding of the anti-CD22 antibody to CD22; and comparing the level of expression of CD22 by the test cell with the level of expression of CD22 by a control cell (e.g., a normal cell of the same tissue origin as the test cell or a cell that expresses CD22 at levels comparable to such a normal cell), wherein a higher level of expression of CD22 by the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of CD22. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of CD22. 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 B cell disorder and/or a B cell proliferative disorder including, but not limited to, 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.

In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-CD22 antibody to CD22 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing CD22 on its surface. In certain embodiments, the method comprises contacting a cell with an anti-CD22 antibody under conditions permissive for binding of the anti-CD22 antibody to CD22, and detecting whether a complex is formed between the anti-CD22 antibody and CD22 on the cell surface. An exemplary assay for detecting binding of an anti-CD22 antibody to CD22 expressed CD22 on the surface of a cell is a "FACS" assay.

Certain other methods can be used to detect binding of anti-CD22 antibodies to CD22. 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-CD22 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-CD22 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-CD22 antibody from any CD22 that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-CD22 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-CD22 antibody after formation of a complex between the anti-CD22 antibody and CD22, 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-CD22 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-CD22 antibody or immunoconjugate thereof under conditions permissive for binding of the immunoconjugate to CD22. "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 B 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 B cell proliferative disorder. In certain embodiments, the cell proliferative disorder is associated with increased expression and/or activity of CD22. For example, in certain embodiments, the B cell proliferative disorder is associated with increased expression of CD22 on the surface of a B cell. In certain embodiments, the B cell proliferative disorder is a tumor or a cancer. Examples of B cell proliferative disorders to be treated by the antibodies or immunoconjugates of the invention include, but are not limited to, 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.

In one aspect, the invention provides methods for treating a B cell proliferative disorder comprising administering to an individual an effective amount of an anti-CD22 antibody or immunoconjugate thereof. In certain embodiments, a method for treating a B cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising an anti-CD22 antibody or anti-CD22 immunoconjugate and, optionally, at least one additional therapeutic agent, such as those provided below. In certain embodiments, a method for treating a cell proliferative disorder comprises administering to an individual an effective amount of a pharmaceutical formulation comprising 1) an immunoconjugate comprising an anti-CD22 antibody and a

cytotoxic agent; and optionally, 2) at least one additional therapeutic agent, such as those provided below.

In one aspect, at least some of the antibodies or immunoconjugates of the invention can bind CD22 from species other than human. Accordingly, antibodies or immunoconjugates of the invention can be used to bind CD22, e.g., in a cell culture containing CD22, in humans, or in other mammals having a CD22 with which an antibody or immunoconjugate of the invention cross-reacts (e.g. chimpanzee, baboon, marmoset, cynomolgus and rhesus monkeys, pig or mouse). In one embodiment, an anti-CD22 antibody or immunoconjugate can be used for targeting CD22 on B cells by contacting the antibody or immunoconjugate with CD22 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 CD22 is human CD22.

In one embodiment, an anti-CD22 antibody or immunoconjugate can be used in a method for binding CD22 in an individual suffering from a disorder associated with increased CD22 expression and/or activity, the method comprising administering to the individual the antibody or immunoconjugate such that CD22 in the individual is bound. In one embodiment, the bound antibody or immunoconjugate is internalized into the B cell expressing CD22. In one embodiment, the CD22 is human CD22, and the individual is a human individual. Alternatively, the individual can be a mammal expressing CD22 to which an anti-CD22 antibody binds. Still further the individual can be a mammal into which CD22 has been introduced (e.g., by administration of CD22 or by expression of a transgene encoding CD22).

An anti-CD22 antibody or immunoconjugate can be administered to a human for therapeutic purposes. Moreover, an anti-CD22 antibody or immunoconjugate can be administered to a non-human mammal expressing CD22 with which the antibody cross-reacts (e.g., a primate, 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-CD20 (e.g., Rituxan®) or anti-VEGF (e.g., Avastin®), wherein the combination therapy is useful in the treatment of cancers and/or B cell disorders such as B cell proliferative disorders including 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.

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. 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-CD22 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-CD22 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-CD22 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-CD22 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-CD22 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 CD22 or that have been engineered to express CD22. Such cells include tumor cells that overexpress CD22 relative to normal cells of the same tissue origin. Such cells also include cell lines (including tumor cell lines) that express CD22 and cell lines that do not normally express CD22 but have been transfected with nucleic acid encoding CD22.

In one aspect, an anti-CD22 antibody or immunoconjugate thereof is tested for its ability to inhibit cell growth or proliferation in vivo. In certain embodiments, an anti-CD22 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 leukemia and lymphoma cell lines, which include without limitation the BJAB-luc cells (an EBV-negative Burkitt's lymphoma cell line transfected with the luciferase reporter gene), Ramos cells (ATCC, Manassas, VA, CRL-1923), Raji cells (ATCC, Manassas, VA, CCL-86), SuDHL-4 cells (DSMZ, Braunschweig, Germany, AAC 495), DoHH2 cells (see Kluin-Neilemans, H.C. et al., Leukemia 5:221-224 (1991), and Kluin-Neilemans, H.C. et al.,

Leukemia 8:1385-1391 (1994)), Granta-519 cells (see Jadayel, D.M. et al, Leukemia 11(1):64-72 (1997)). 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-CD22 antibody is tested for its antigen binding activity. For example, in certain embodiments, an anti-CD22 antibody is tested for its ability to bind to CD22 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 murine 10F4.4.1 antibody, humanized 10F4v1 antibody, humanized 10F4v3 antibody and/or murine 5E8.1.8 antibody for binding to CD22. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by murine 10F4.4.1 antibody, humanized 10F4v1 antibody, humanized 10F4v3 antibody and/or murine 5E8.1.8 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 CD22 is incubated in a solution comprising a first labeled antibody that binds to CD22 (e.g., murine 10F4.4.1 antibody, humanized 10F4v1 antibody, humanized 10F4v3 antibody and/or murine 5E8.1.8 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to CD22. The second antibody may be present in a hybridoma supernatant. As a control, immobilized CD22 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 CD22, excess unbound antibody is removed, and the amount of label associated with immobilized CD22 is measured. If the amount of label associated with immobilized CD22 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 CD22. In certain embodiments, immobilized CD22 is present on the surface of a cell or in a membrane preparation obtained from a cell expressing CD22 on its surface.

In one aspect, purified anti-CD22 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 murine anti-human CD22 monoclonal antibody

Murine monoclonal antibodies capable of specifically binding human CD22 was prepared. BALB/c female mice, age six weeks, were immunized in their foot pads with purified human CD22 his-8 tagged extracellular domain lacking domains 3 and 4 (SEQ ID NO:30 (ECD) plus the sequence GRAHHHHHHHH at the C-terminus) or CD22 his-8 tagged extracellular domain comprising domains 1-7 (SEQ ID NO:28 (ECD) plus the above His sequence tag) in Ribi's adjuvant. Subsequent injections were performed in the same manner at one and three weeks after the initial immunizations. Three days after the final injection, the inguinal and popliteal lymph nodes were removed and pooled, and a single cell suspension was made by passing the tissue through steel gauze. The cells were fused at a 4:1 ratio with mouse myeloma such as P3X63-Ag8.653 (ATCC CRL 1580) in high glucose (DMEM) containing 50% w/v polyethylene glycol 4000. The fused cells were then plated at a density of 2x105 per well in 96 well tissue culture plates. After 24 hours HAT selective medium (hypoxanthine/aminopterin/thymidine, Sigma, #H0262) was added. Fifteen days after the fusion, supernatants of growing cells were tested for the presence of antibodies specific for human CD22 using an enzyme-linked immunosorbent assay (ELISA).

The murine anti-human CD22 10F4.4.1 (mu 10F4) and 5E8.1.8 (mu 5E8) monoclonal antibodies were selected for further study based on cell-based assays and plate assays which showed the antibodies to bind specifically to human CD22. The assays are described in the following paragraphs.

ELISA-based assays: Anti-CD22 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 CD22 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 □l 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.

Example 2: FACS based assays for analysis of ant-human CD22 monoclonal antibodies (MAbs).

CHO cells expressing human CD22 on their surface were incubated with anti-CD22 hybridoma supernatant in 100 µl FACS buffer (0.1 % BSA, 10 mM sodium azide in PBS, pH 7.4) for 30 minutes at 4°C followed by one wash with FACS buffer. The amount of anti-CD22 binding was determined by incubating an aliquot of the antibody/cell mixture with a polyclonal FITC conjugated goat or rabbit anti-mouse IgG (Accurate Chem. Co., Westbury, NY) (for murine test antibodies) or goat or rabbit anti-human IgG (for humanized antibodies) for 30 minutes at 4°C followed by three washes with FACS buffer.

Example 3: Preparation of Humanized anti-CD22 antibodies

Humanized 10F4 antibodies were generated wherein hypervariable region (HVR) amino acid residues (interchangeably referred to as complementarity determining regions or CDRs) were modified via site-directed mutagenesis (Kunkel et al., Methods Enzymol. (1987), 154:367-382) to arrive at two variants, humanized 10F4v1 and humanized 10F4v2 (also referred to herein as "10F4v1," "hu10F4v1," "10F4v2," or "hu10F4v2," respectively). A third version, humanized 10F4v3 ("10F4v3" or "hu10Fv3"), used in some studies disclosed herein has the same light and heavy chain amino acid sequences for the mature protein as hu10F4v2, but comprises a different signal sequence in the vector used for protein expression.

Humanization of the murine 10F4 antibody was preformed as disclosed herein. Briefly, the hypervariable regions of the light and heavy chains of murine 10F4 were cloned into modified consensus framework sequences to generate the light and heavy chain variable regions amino acid sequences shown in Figures 2A and 2B. Alternative light and heavy

chain framework sequences that may be used as framework sequences of antibodies of the invention are shown in Figures 3 and 4.

A monovalent Fab-g3 display vector (pV0350-2B) phagemid having two open reading frames under control of the *pho*A promoter, essentially as described in Lee *et al., J. Mol. Biol.* 340:1073-93 (2004), was used in the humanization of the 10F4 antibody. The first open reading frame comprised the E. coli heat stable STII signal sequence for protein secretion fused to the VL and CH1 domains of the acceptor light chain sequence. The second open reading frame comprised the STII signal sequence fused to the VH and CH1 domains of the acceptor heavy chain sequence followed by a truncated minor phage coat protein P3.

The VH and VL domains from murine 10F4 (SEQ ID NOs:89 and 90, respectively) were aligned with the human subgroup III consensus VH (huIII) domain (SEQ ID NO:24) and human consensus kappa1 (huK1) domain (SEQ ID NO:25), respectively. The amino acid sequences of the hypervariable regions (HVRs, interchangeably referred to herein as complimentary determining regions (CDRs)) of the murine anti-human CD22 MAb 10F4 were inserted into consensus framework sequences as follows. The light chain HVRs (HVR-L1 (Kabat positions 24-34), HVR-L2 (Kabat positions 50-56), and HVR-L3 (Kabat positions 89-97) of the mu 10F4 antibody were engineered into a human kappa I (huKI) consensus sequence antibody framework to produce the humanized 10F4v1 light chain (SEQ ID NO:17, Fig. 2B). The heavy chain HVRs (HVR-H1 (Kabat positions 26-35), HVR-H2 (Kabat positions 49-65), and HVR-H3 (Kabat positions 95-102) of the mu 10F4 antibody were engineered into a modified human subgroup III (humIII) consensus VH domain which differs from the humIII sequence at three positions: R71A, N73T, and L78A were used (see Carter et all, Proc. Natl. Acad. Sci. USA 89:4285 (1992)) to produce the humanized 10F4v1 heavy chain variable region (SEQ ID NO:16, Fig. 2A). Genetic engineering of HVRs into the acceptor frameworks was performed by Kunkel mutagenesis using a separate oligonucleotide for each hypervariable region. The sequence of each clone was determined by standard DNA sequencing techniques. Hypervariable regions and framework regions shown in Figures 2A and 2B are numbered according to Kabat numbering (Kabat et al. (1991), supra). The light and heavy chains were sequenced and the amino acid sequences of the variable regions (including HVRs and framework regions (FRs)) of the huKI, the humIII, murine 10F4, humanized 10F4v1 and humanized 10F4v2 are

shown in Figures 2A and 2B. Humanized 10F4v3 antibody has the identical amino acid sequence as 10F4v2.

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)

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 (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 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 (ie., 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.

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.

Example 4: Variable Region Sequence Determination

The nucleic acid and amino acid sequences of the murine and humanized 10F4 monoclonal antibodies were determined by standard procedures. Total RNA was extracted from hybridoma cells producing the mouse anti-human CD22 10F4.4.1 monoclonal antibodies using the RNeasy® Mini Kit (Qiagen, Germany). The variable light (VL) and variable heavy (VH) domains were amplified using RT-PCR with degenerate primers. The forward primers were specific for the N-terminal amino acid sequences of the VL and VH regions of the antibody. Respectively, the light chain and heavy chain reverse primers were designed to anneal to a region in the constant light (CL) and constant heavy domain 1 (CH1), which is highly conserved across species. Amplified VH andVL were cloned into a pRK mammalian cell expression vector (Shields et al., J. Biol. Chem. 276:659-04 (2000)). The polynucleotide sequence of the inserts was determined using routine sequencing methods. The amino acid sequences of the murine chimeric 10F4 and humanized 10F4v1 and humanized 10F4v2 light and heavy chain variable regions are shown in Figures 2A and 2B.

Humanized 10F4v1 was further modified at HVR-L1 position 28 (N28) (SEQ ID NO:9) (see Figure 2B. The asparagine residue at that position was replaced with a valine residue (N28V) to generate HVR-L1 (SEQ ID NO:10) for the hu10F4v2 and hu10F4v3

variants, which showed improved binding affinity. These variants comprise the same variable and constant domain sequences of the mature antibody and differ only in a signal sequence not found in the mature antibody of the invention.

Additional amino acid sequence modifications were generated at one or both of amino acids Asn28 (N28) and/or Asn30 (N30) of the HVR-L1 hypervariable region (see Figure 2B) of hu10F4v1. Because N28 and N30 are possible sites for deamination, amino acid changes at these sites were tested. For example, Asparagine at position 28 (N28) was replaced alternatively with A, Q, S, D, V, or I, and Asparagine at position 30 (N30) was replaced alternatively with A or Q. Amino acid sequence changes in the HVR-L1 domain according to the invention are provided in Table 2 along with their binding affinities as tested by competition analysis in a phage ELISA assay (IC50) using standard procedures.

Table 2

Substitution Variants of hu 10F4v1 antibody

Amino Acid Change In HVR-L1 Figure 2B	HVR-L1 SEQ ID NO	Binding Affinity (nM)
No change (N28, N30)	9	8
N28A, N30	19	8
N28Q, N30	20	7.3
N28S, N30	21	12
N28D, N30	22	12
N28V, N30	10	7.3
N28I, N30	23	9.8
N28, N30A	32	7.7
N28, N30Q	33	10

For generation of full-length human IgG1 versions of humanized 10F4 antibody, the heavy and light chains are subcloned separately into previously described pRK plasmids (Gorman, C.M. et al. (1990), DNA Protein Eng. Tech. 2: 3). Appropriate heavy and light chain plasmids (depending upon the sequence change(s) desired) are cotransfected into an adenovirus-transformed human embryonic kidney cell line, known as 293 (Graham, F.L. et

al. (1977), J. Gen. Virol. 36: 59), using a high efficiency procedure (Graham et al., supra & Gorman, C.M., Science 221: 551). Media is changed to serum free and harvested daily for up to 5 days. Antibodies are purified from the pooled supernatants using protein A-Sepharose CL-4B (Pharmacia). The eluted antibody is buffer exchanged into PBS by G25 gel filtration, concentrated by ultrafiltration using a Centriprep-30 or Centricon-100 (Millipore), and stored at 4°C. The concentration of antibody is determined using total IgG-binding ELISA.

Exemplary heavy chain IgG1 constant domains according to the invention are depicted in Figure 5A. An exemplary human light chain κ constant domain comprises, for example,
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHDVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:37). The full length amino acid sequence of h10F4v2 is shown in Figure 5B in which the constant regions of the light and heavy chains are indicated by underlining. The h10F4v1, v2, and v3 antibodies are IgG1 isotype.

Characterization of anti-CD22 antibodies

Example 5: Epitope mapping:

The epitopes of CD22 to which 10F4.4.1 and 5E8.1.8 antibodies bound were determined according to the following procedures. CD22 sequences lacking various of the seven immunoglobulin-like domains of the major CD22 isoform (CD22beta) were cloned and transformed into cells for stable expression. For example, CD22 variants lacking domain 1 (Δ1), domain 2 (Δ2), or domains 3 and 4 (Δ3,4) were cloned, transformed into CHO cells, and expressed on the cells. Control cells expressed CD22beta. Deletions were performed using Stratagene QuikChange XL™ reagent kit. Deletion of domain 1 was performed by deletion of amino acids 22-138; deletion of domain 2 was performed by deletion of amino acids 139-242; and deleted domains 3 and 4 were available as the minor isoform CD22alpha (deletion of amino acids 241-417). All amino acid numbers refer to the numbering of full length precursor CD22beta by Wilson, G.L. et al. (see Figure 1 in Wilson, G.L. et al., J. Exp. Med. 173:137-146 (1991)). Figure 14 is a diagram of the deleted

domains. Binding was determined by flow cytometry using an isotype control. Binding of 10F4.4.1 was detected using goat anti-mouse IgG Alexa 488. Binding of 5E8.1.8 was detected using biotinylated goat anti-mouse IgG plus streptavidin PE. An adverse affect on the binding of murine 10F4.4.1 or murine 5E8.1.8 antibodies in the absence of particular ECD domains indicated that the antibody bound those domains. Murine 10F4.4.1 and 5E8.1.8 showed the same binding characteristics under these conditions. Neither bound CD22 lacking domain 1 or domain 2, and both bound CD22 comprising domains 1 and 2, but lacking domains 3 and 4. Using this method, it was determined that 10F4.4.1 and 5E8.1.8 bind to domains 1 and 2 of human CD22, within the sequence from amino acid 22 to amino acid 240 of SEQ ID NO:27 (see Wilson, G.L. et al., (1991) supra).

Example 6: Characterization of Binding Affinity to Soluble Antigen

The binding affinity of murine and humanized 10F4 antibody for soluble CD22 extracellular domain (ECD) was determined by surface plasmon resonance measurement using a BIACORE® 3000 system (Biacore, Inc., Piscataway, NJ). Briefly, carboxymethylated dextran biosensor chips (CM5, Biacore Inc.) were activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. These activated chips were coated with anti-CD22 IgG1 antibody 10F4 (murine or humanized) by dilution to 5 µg/ml with 10mM sodium acetate, pH 4.8, before injection at a flow rate of 5µl/minute to achieve approximately 500 response units (RU) of coupled antibody. Next, 1M ethanolamine was injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of human CD22-beta-ECD-His tagged soluble antigen (approximately 500 nM to approximately 7.8 nM) were injected in PBS with 0.05% Tween 20 at 25°C at a flow rate of 30 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) were calculated using a simple one-to-one Langmuir binding model (BIAevaluation Software version 3.2). The equilibrium dissociation constant (Kd) was calculated as the ratio k_{off}/k_{on}. Anti-CD22 antibody, RFB4, was used as a control (Chemicon International, Inc., Temecula, CA, catalog no. CBL147). The results of this experiment are shown in Table 2 below.

TABLE 2

Anti-CD22 Binding Affinity to Soluble Human CD22 (BIACORE® analysis)

Clone	$k_{on}/10^5$	$k_{off}/10^{-4}$	Kd (nM)
Murine 10F4	0.19	2.8	15
Chimeric 10F4	0.26	4.2	16
Humanized 10F4v1	0.18	3.5	19
Humanized 10F4v2	0.32	2.5	7.8
Control RFB4	0.33	1.4	4.2

Example 7: Characterization of Binding Affinity to Cell Surface Antigen

The binding affinity of murine 10F4.4.1 and humanized 10F4v1 and 10F4v2 for human and cynomolgus monkey (cyno) CD22 expressed on the surface of CHO cells was examined using a competition assay. Briefly, CHO cells stably expressing full length human CD22 (SEQ ID NO:27) or cynomolgus monkey (cyno) CD22 (SEQ ID NO:31). Anti-CD22 antibody (murine or humanized 10F4v1 or v2) was iodinated with Iodogen® [^{125}I] reagent to a specific activity of approximately 10 μCi/μg. A cell-based, competitive binding assay was performed using serially diluted, unlabeled anti-CD22 antibody. Antibodies were allowed to bind to the cells for 4 hours at 4°C. Binding affinity, K_D, of the antibodies was determined in accordance with standard Scatchard analysis performed utilizing a non-linear curve fitting program (see, for example, Munson et al., Anal Biochem, 107: 220-239, 1980). The results of this experiment are shown in Table 3 below.

TABLE 3

10F4 MAb Binding Affinity for Human and Cyno CD22

Antibody	Human CD22 Kd (nM)	Cyno CD22 Kd (nM)
Mu 10F4.4.1	2.4	2.3
Hu 10F4v1*	1.1, 1.7	1.4, 1.8
Hu 10F4v2	1.6	2.1

*repeated assays

The results indicate that murine and humanized 10F4 bind human and cyno CD22 expressed on the surface of CHO cells with approximately equivalent affinity.

Example 8: Production of Anti-CD22 Antibody Drug Conjugates

Anti-CD22 ADCs were produced by conjugating anti-CD22 antibodies RFB4, murine 5E8, murine 10F4, humanized 10F4v1, humanized thioMAb 10F4v1 (thio-10F4v1), humanized 10F4v2, and humanized 10F4v3 to the following drug-linker moieties: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE and MC-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 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. Other useful linkers for the preparation of ADCs include, without limitation, BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate), and including bis-maleimide reagents: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, and $BM(PEO)_4$.

TABLE 3

10F4 MAb Binding Affinity for Human and Cyno CD22

Antibody	Human CD22 Kd (nM)	Cyno CD22 Kd (nM)
Mu 10F4.4.1	2.4	2.3
Hu 10F4v1*	1.1, 1.7	1.4, 1.8
Hu 10F4v2	1.6	2.1

*repeated assays

The results indicate that murine and humanized 10F4 bind human and cyno CD22 expressed on the surface of CHO cells with approximately equivalent affinity.

Example 8: Production of Anti-CD22 Antibody Drug Conjugates

Anti-CD22 ADCs were produced by conjugating anti-CD22 antibodies RFB4, murine 5E8, murine 10F4, humanized 10F4v1, humanized thioMAb 10F4v1 (thio-10F4v1), humanized 10F4v2, and humanized 10F4v3 to the following drug-linker moieties: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE and MC-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 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. Other useful linkers for the preparation of ADCs include, without limitation, BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate), and including bis-maleimide reagents: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, and $BM(PEO)_4$.

Anti-CD22 ADCs are also produced by conjugation to lysine residues of the antibody. Lysines of the antibody are converted to sulfhydryl groups using, for example, Traut's reagent (Pierce Chemical Co.) as disclosed herein. The resultant sulfhydryl groups are reactive with linkers or linker drug molecules for the preparation of ADCs.

Alternatively, ADC's are produced by reacting a lysine on an anti-CD22 antibody with the linker, SPP (N-succinimidyl 4-(2'-pyridyldithio) pentanoate, which may be already attached to a drug molecule or may be subsequently reacted with a drug molecule, such as a maytansinoid. For example, an antibody is modified by reaction with SPP, followed by conjugation with f as disclosed in Wang, L. et al., Protein Science 14:2436-2446 (2005), which reference is hereby incorporated by reference in its entirety. Lysine residues on an anti-CD22 antibody may also be reacted with the linker, SMCC (Pierce Chemical Co.), at pH 7-9 such that the amine-reactive N-hydroxysuccinimide (NHS ester) of SMCC forms a stable amide bond with the antibody. The sulfhydryl-reactive maleimide group of SMCC is reacted with the sulfhydryl group of DM1 at pH 6.5-7.5 (see Pierce Chemical Co., piercenet.com) to form the ADC. Lysine or cysteine residues are reacted with linker-drug to produce ADCs comprising an average drug load of approximately 1-8 linker drug molecules per antibody, alternatively 1-6, 1-4, 1-3 or 1-2 linker drug molecules per antibody.

ADCs anti-CD22(RFB4)-SMCC-DM1 and anti-GP120-SMCC-DM1 were prepared according to this method, where RFB4-smcc-DM1 was prepared at low (1.95), medium (3.7) and high (6.75) drug loads. Anti-GP120-smcc-DM1 was prepared at high (6.1) drug load. These ADCs were shown to be efficacious in vivo, as shown in Example 9 and Table 9, herein below.

Example 9: Efficacy of Anti-CD22 Antibody Drug Conjugates

In vitro studies of efficacy determinants.

The determinants of anti-CD22 ADC (or TDC) efficacy in a lymphoma cell line were determined. It is known that CD22 expressed on the surface of B cells is internalized upon binding of its ligand(s) or antibodies (Sato, S. et al., Immunity 5:551-562 (1996)). To test whether and how the level of B cell surface expression of CD22 and/or internalization of CD22 affect efficacy, the following in vitro studies were performed.

Surface expression of human CD22 on multiple lymphoma cell lines. Nineteen lymphoma cell lines expressing varying amounts of CD22 on their surface were cultured and harvested in log phase growth. Cells were resuspended in FACS wash buffer (PBS; 0.5% bovine serum albumin; 0.1% sodium azide) containing 100 µg/ml each normal mouse IgG and normal human IgG and maintained on ice. Approximately 1 x 10^6 cells/100 µl were stained with anti-huCD22 APC (mIgG1, clone RFB4, Southern Biotech #9361-11) or murine IgG1 APC isotype (BD Pharmingen #555751) for 30 minutes on ice. Dead cells were stained with 7-AAD (BD Pharmingen #559925). Data were acquired on a BD FacsCalibur™ flow cytometer and analyzed with FlowJo™ software. The IC50 determination for hu10F4v3-SMCC-DM1 or each free drug (DM1, MMAF, or MMAE) were determined by culturing lymphoma cells as above, harvesting the cultured cells in log phase and seeding 5,000 cells in 90 µl culture medium per well in 96 well plate. ADC and free drug were diluted serially within the detection range (starting at 300 µg/ml for ADC, or 90 nM for free drug and diluting to essentially zero assay target). Aliquots of 10 µl diluted ADC or free drug were added to replicate wells containing cells and incubated for 3 days at 37 °C. To each well, 100 µl CellTiter Glo™ was added and incubated for 30 min. Chemiluminescence was detected and data were analyzed using Prism™ software. The results are shown in Figure 6A, in which high surface CD22 levels correlate with low IC50 (higher efficacy) of hu10F4v3-SMCC-DM1. Figure 6C indicates that a stronger correlation exists between the intrinsic sensitivity of the cells to free drug and the IC50 of the ADC.

Internalization of hu10F4v3-SMCC-DM1 was determined by FACS assay. Briefly, lymphoma cells were stained by standard FACS techniques with CD22-FITC (RFB4) in the presence of hu10F4v3-SMCC-DM1 and incubated on ice for 20-30 minutes. To determine CD22 levels on the cell surface after the initial staining, cells were washed in cold RPMI/10% FBS media and 200 µl pre-warmed RPMI/10% FBS was added and incubated at 37 °C for 15 minutes. 80 µl staining buffer and 20µl heat-inactivated normal mouse serum (HI NMS) were added, followed by incubation on ice for 15 minutes. Anti-DM1-Alexa-647 was added, incubated on ice for 20-30 minutes and cells were washed and fixed with 200 µl PBS/1% paraformaldehyde prior to FACS analysis. To determine surface and internal staining of CD22 after the initial staining, cells were washed with cold RPMI/10% FBS, pre-warmed RPMI/10% FBS was added and the cells incubated for 15 minutes at 37 °C. Cells were then washed with FACS Wash and fixed with Fix Reagent A (Dako™ #k2311)

at room temperature for 15 minutes, and the step was repeated with Fix Reagent B (Dako™). Staining buffer and HI NMS were added and the cell mixture was incubated on ice for 15 min. Fix Reagent B was added, followed by anti-DM1-Alexa-647 and incubated at room temperature for 20-30 minutes. Cells were washed in FACS Wash and fixed in PBS/1% paraformaldehyde. FACS analysis was performed on each cell mixture (surface, surface post-internalization, and internal staining) using a BD FacsCalibur™ flow cytometer and analyzed with FlowJo™ software. The results are shown in Figure 6B in which high amounts of internalized DM1 correlated with low IC50 (high efficacy); and in Figure 6D in which internalized DM1 is visualized by fluorescent microscopy.

In vivo efficacy studies.

To test the efficacy of toxin-conjugated or unconjugated anti-CD22 monoclonal antibodies for the ability to reduce tumor volume in vivo, the following protocol was employed.

SCID mice were each inoculated subcutaneously in the flank with 2 x 10^7 a human B-cell lymphoma cell line. The human cell lines included human Burkitt lymphoma cell lines Daudi, Ramos, and Raji cells (available from the American Type Culture Collection, Manassas, VA, USA), and other B-cell lines including U-698-M cells and Su-DHL-4 cells (available from DSMZ, Braunschweig, Germany; Su-DHL-4 cells were transfected with the luciferase reporter gene), DoHH2 cells (Kluin-Neilemans, H.C. (1991), supra), and Granta-519 (Mantle cell lymphoma cells, Jadayel, D.M. et al., Leukemia 11(1):64-72 (1997)), and BJAB-luc cells (BJAB human B-cell lymphoblastoid cell line which expresses reporter gene luciferase. When the tumors reached a mean tumor volume of between 100-200 mm3, the mice were divided into groups, and treated on day 0 by intravenous injection with toxin-conjugated antibody or unconjugated antibody as shown in Tables 4-16, below.

Anti-CD22 maytansine drug conjugates reduce B-cell tumor volume

Sixty-five SCID mice were injected with 2x10^7 BJAB-luc cells subcutaneously in a volume of 0.2 ml per mouse in the flank. Cells were suspended in HBSS. When the mean tumor size reached 100-200mm^3, mice were randomly grouped into four groups of 9 mice each and given a single I.V. treatment (via the tail vein) of the anti-CD22 or control antibody indicated in Table 4, below.

Table 4

In Vivo Tumor Volume Reduction
Antibody Administration

Antibody administered	TI	PR	CR	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)	Drug ratio (Drug moieties/Ab)
anti-Her2-smcc-DM1	9/9	0	0	4.2	200	3.2
mu10F4-smcc-DM1	9/9	2	0	3.0	200	4.6
hu10F4v2-smcc-DM1	9/9	0	0	3.4	200	4.0
hu10F4v1	9/9	0	0	3.4	---	---

"TI"- tumor incidence at the last time point of each group; the numerator refers to the number of tumor-bearing animals and the denominator refers to total number of animals. "PR" refers to the number of animals with tumor regressed 50-99% from its initial volume. "CR" refers to the number of animals attaining complete remission.

Mean tumor volume was monitored in each treatment group for 32 days post-antibody injection. Tumor measurements were taken with calipers. Efficacy of the toxin-conjugated anti-CD22 antibodies was determined by comparison to the control and unconjugated antibodies. The results are shown in Figure 7A. The murine and humanized 10F4v1-smcc-DM1 monoclonal antibodies significantly slowed tumor growth relative to unconjugated anti-CD22 antibody and non-specific control antibody.

Using the same protocol as above, an assay was performed comparing toxin-conjugated humanized 10F4v2 to toxin-conjugated murine and naked humanized antibody as indicated in Table 5, below.

Table 5

In Vivo Tumor Volume Reduction
Antibody Administration

Antibody administered	TI	PR	CR	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)	Drug ratio (Drug moieties/Ab)
anti-Her2-smcc-DM1	9/9	0	0	4.2	200	3.2
mu10F4-	7/9	1	2	4.7	200	2.9

smcc-DM1						
hu10F4v2-smcc-DM1	8/9	1	1	4.5	200	3.0
hu10F4v2	9/9	0	0	4.5	---	---

"TI"- tumor incidence at the last time point of each group; the numerator refers to the number of tumor-animals in the study group and the denominator refers to total number of animals. "PR" refers to the number of animals with tumor regressed 50-99% from its initial volume. "CR" refers to the number of animals attaining complete remission.

Mean tumor volume was monitored in each treatment group for 32 days post-antibody injection. Tumor measurements were taken with calipers. Efficacy of the toxin-conjugated anti-CD22 antibodies was determined by comparison to the control and unconjugated antibodies. The results are shown in Figure 7B. The murine 10F4-smcc-DM1 and humanized 10F4v2-smcc-DM1 monoclonal antibodies significantly slowed tumor growth relative to unconjugated anti-CD22 antibody and non-specific control antibody.

Anti-CD22 antibody was conjugated to DM1 via the spp linker or the smcc linker according to conjugation methods disclosed herein. The naked anti-CD20 antibody was used as a positive control and the toxin conjugates, anti-HER2-spp-DM1 and anti-HER2-smcc-DM1, were used as negative controls. Eighty SCID mice were injected with 2x10^7 BJAB-luc cells subcutaneously in a volume of 0.2ml per mouse in the flank. Cells were suspended in HBSS. When the mean tumor size reached 100-200mm^3, the mice were randomly grouped into six groups of 10 mice each and intravenous injection of test or control antibodies was performed. Doses were repeated once each week for a total of three doses. See Table 6.

Table 6
In Vivo Tumor Volume Reduction
Antibody Administration

Antibody administered	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)
anti-Her2-spp-DM1 *	4	214
anti-Her2-smcc-DM1 **	6.9	405
anti-CD22-spp-DM1 *	5	214
anti-CD22-spp-DM1	2.5	107

anti-CD22-smcc-DM1 **	10	405
naked anti-CD22	10	---

* Matched drug load
** Matched drug load

Mean tumor volume was monitored twice each week for 3 weeks and then once each week thereafter for a total of 8 weeks. Changes in tumor volume over time (Figure 7C) show that the anti-CD22-spp-DM1 dosed at 214 and 107 μg/m^2 DM1 and anti-CD22-smcc-DM1 dosed at 405 μg/m^2 showed robust and comparable anti-tumor activity in BJAB-luc xenograft tumors. All anti-CD22 ADC groups showed complete responses.

The anti-CD22 antibodies, RFB4, 5E8, and 7A2 were conjugated to DM1 via the smcc linker according to conjugation methods disclosed herein. The toxin conjugate, anti-HER2-smcc-DM1 (referred to interchangeably herein as HER-smcc-DM1 or HER2-smcc-DM1), was used as negative control.

The ability of these antibodies to reduce tumor volume in various xenografts in SCID mice was examined. The human B-cell lymphoma cell lines used to generate xenograft tumors in mice were Ramos cells and BJAB-luc cells. For each xenograft, SCID mice were injected with 5x10^6 human B-cell lymphoma Ramos cells subcutaneously in a volume of 0.1ml per mouse in the flank (or 2x10^7 BJAB-luc cells in 0.2ml). Cells were suspended in HBSS. When the mean tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous injection of test or control antibody. DM1 drug loading was normalized to 200 $\mu g/m^2$ for each group to provide the dose of DM1 administered. Mean tumor volume was monitored twice each week for 4 weeks. The results are shown below in Tables 7 and 8 and plotted in Figures 8A and 8B, respectively.

Table 7

In Vivo Tumor Volume Reduction, Ramos Xenograft
Antibody Administration

Antibody administered	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)

anti-HER2-smcc-DM1	4.2	200
Anti-CD22(7A2)-smcc-DM1	3.8	200
anti-CD22(5E8)-smcc-DM1	3.8	200
Anti-CD22(RFB4)-smcc-DM1	3.2	200

Table 8

In Vivo Tumor Volume Reduction, BJAB-luc Xenograft Antibody Administration

Antibody administered	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)	Drug ratio (Drug moieties/Ab)
anti-HER2-smcc-DM1	4.2	200	3.2
Anti-CD22 (7A2)-smcc-DM1	3.8	200	3.6
Anti-CD22(5E8)-smcc-DM1	3.8	200	3.6
Anti-CD22(RFB4)-smcc-DM1	3.2	200	4.25

These results show that anti-CD22-smcc-DM1 antibody drug conjugates significantly reduce B-cell tumor volume in Ramos; and BJAB-luc xenografts relative to control antibody or naked anti-CD22 antibody.

The affect of antibody drug load (average number of drug molecules conjugated per antibody in a population of antibodies) on the ability of anti-CD22-smcc-DM1 antibody drug conjugates to reduce tumor volume in BJAB-luc SCID mouse xenografts was examined. One hundred forty SCID mice were injected with 2x10^7 BJAB-luc cells subcutaneously in a volume of 0.2ml per mouse in the flank. Cells were suspended in HBSS. When the mean tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous

injection of test or control antibody. Populations of anti-CD22(RFB4)-smcc-DM1 having relative low, medium or high drug loads (average drug loads 1.95, 3.7, or 6.75 conjugated DM1 molecules per antibody, resptectively) were administered as the test antibodies. Naked RFB4 antibody and anti-GP120-smcc-DM1 (high drug load) were the controls. The doses of antibody drug conjugates (test and control) were normalized to a dose of 5mg/kg protein level. Linker conjugate attachment to the antibodies was via lysine residues. See Table 9.

Table 9
In Vivo Tumor Volume Reduction, BJAB-luc Xenograft
Anti-CD22(RFB4)-smcc-DM1 Administration

Antibody administered	Dose Ab (mg/kg)	Dose DM1 ($\mu g/m^2$)	Drug ratio (Drug moieties/Ab)
anti-CD22(RFB4) (naked antibody)	10	--	--
anti-CD22(RFB4)-smcc-DM1 (low)	5	144	1.95
anti-CD22(RFB4) - smcc-DM1 (medium)	5	273	3.7
anti-CD22(RFB4)-smcc-DM1 (high)	5	497	6.75
anti-GP120-smcc-DM1 (high)	5	449	6.1

When dosed at a matching protein level (5 mg/kg), anti-CD22(RFB4)-smcc-DM1 loaded with a high drug load (6.75 DM1 molecules per antibody molecule) reduced tumor volume slightly more than the antibody drug conjugate with a medium load of 3.7, whereas the affects of the antibody drug conjugate with a low drug load was not different from control conjugate or naked antibody. The results are plotted in Figure 9.

Anti-CD22 auristatin drug conjugates reduce B-cell tumor volume

The affect of anti-CD22 auristatin MMAF drug conjugates on tumor volume in mouse xenografts was examined. Anti-CD22(RFB4) and control antibody anti-GP120 were conjugated to MMAF via a MC-vcPAB linker or a MC linker according to methods disclosed herein. SCID mice were injected with 5x10^6 Ramos cells subcutaneously in a volume of 0.2ml per mouse in the flank. Cells were suspended in HBSS. When the mean

tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous injection of test or control antibody. Drug dose, drug load (drug ratio) and antibody dose administered to the mice are shown in Table 10.

Table 10
In Vivo Tumor Volume Reduction, Ramos Xenograft
Anti-CD22(RFB4) MMAF Conjugate Administration

Antibody administered	Dose MMAF ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug moieties/Ab)
anti-CD22(RFB4)-MCvcPAB-MMAF	405	6.6	4.2
anti-CD22(RFB4)-MC-MMAF	405	6.9	4.0
anti-GP120-MCvcPAB-MMAF	405	5.8	4.8
anti-GP120-MC-MMAF	405	5.9	4.7

Anti-CD22-MC-MMAF showed comparable activity compared to anti-CD22-MC-vc-PAB-MMAF in Ramos RA1 xenografts. The results are plotted in Figure 10.

The affect of anti-CD22 auristatin MMAE and DM1 drug conjugates on tumor volume in mouse xenografts was examined. Anti-CD22(RFB4) and control antibody anti-GP120 were conjugated to MMAE via a MC-vcPAB linker or a MC linker or to DM1 via a smcc linker according to methods disclosed herein. SCID mice were injected with 5x10^6 Ramos cells subcutaneously in a volume of 0.1 ml per mouse in the flank. Cells were suspended in HBSS. PBS was administered as a control. When the mean tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous injection of test or control antibody. Drug dose, drug load (drug ratio) and antibody dose administered to the mice are shown in Table 11.

Table 11
In Vivo Tumor Volume Reduction, Ramos Xenograft
Anti-CD22(RFB4) MMAE and DM1 Conjugate Administration

Antibody administered	Dose MMAE or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug moieties/Ab)
Anti-GP120-smcc-	405	6.7	4.1

DM1			
Anti-CD22(RFB4)-smcc-DM1	405	6.5	4.25
Anti-GP120-MCvcPAB-MMAE	405	6.0	4.7
antiCD22(RFB4)-MCvcPAB-MMAE	405	6.3	4.5
PBS	--	--	--

Anti-CD22-MCvcPAB-MMAE showed potent anti-tumor activity in Ramos RA1 xenografts. The anti-CD22-MCvcPAB-MMAE showed superior activity compared to antiCD22-smcc-DM1. The ADC control, anti-GP120-MCvcPAB-MMAE, did not show significant activity. The results are plotted in Figure 11.

The affect of anti-CD22 auristatin MMAF and DM1 drug conjugates on tumor volume in mouse xenografts was examined. Anti-CD22 hu10F4v2-MC-MMAF, hu10F4v2-smcc-DM1 and thio-10F4v1-MC-MMAF were administered and compared for affect on tumor volume. Control antibodies were anti-Her2-MC-MMAF and anti-Her2-smcc-DM1. SCID mice were injected with 2x10^7 BJAB-luc cells subcutaneously in a volume of 0.2ml per mouse in the flank. Cells were suspended in HBSS. When the mean tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous injection of test or control antibody. "Thio" refers to a thioMab, as disclosed herein, in which the linker-drug moiety is conjugated to the antibody via a cysteine engineered site on the antibody. Drug dose, drug load (drug ratio) and antibody dose administered to the mice are shown in Table 12.

Table 12
In Vivo Tumor Volume Reduction, BJAB-luc Xenograft
Hu10F4 MMAF and DM1 Conjugate Administration

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug moieties/Ab)
Anti-Her2-MC-MMAF	100	1.1	6.3
Hu10F4v2-MC-MMAF	100	2.0	3.4
Hu10F4v2-MC-MMAF	50	1.0	3.4
Thio-hu10F4v1-MC-MMAF	100	4.6	1.5

Thio-hu10F4v1-MC-MMAF	50	2.3	1.5
Anti-Her2-smcc-DM1	200	4.2	3.2
Hu10F4v2-smcc-DM1	200	4.5	3.0
Hu10F4v2-smcc-DM1	100	2.3	3.0

Hu10F4v2 ADCs showed potent anti-tumor activity in BJAB-luc xenografts. The results are plotted in Figure 12.

Using procedures as disclosed in the above experiments, hu10F4v3 –smcc-DM1 and –MC-MMAF ADC efficacy in different xenografts at different doses was examined. Xenografts of SuDHL4-luc, DoHH2, and Granta-519 xenografts were prepared as disclosed herein, above. When the tumor size reached 100-200mm^3, the mice were randomly grouped into groups of 8-10 mice each, and each mouse was given a single intravenous injection of test or control antibody. Drug dose, drug load (drug ratio) and antibody dose administered to the mice are shown in Tables 13A-13C and the results are shown in Figures 13A-13C.

Table 13A
In Vivo Tumor Volume Reduction,
Hu10F4v3 MMAF and DM1 Conjugate Administration
In SuDHL-4-luc Xenografts

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
Anti-Her2-smcc-DM1	600	11.9	3.3
Hu10F4v3-smcc-DM1	600	13.6	2.9
Hu10F4v3-smcc-DM1	300	6.8	2.9
Anti-Her2-MC-MMAF	600	9.9	4.0
Hu10F4v3-MC-MMAF	600	13.3	3.0
Hu10F4v3-MC-MMAF	300	6.6	3.0

Table 13B
In Vivo Tumor Volume Reduction,
Hu10F4v3 MMAF and DM1 Conjugate Administration
In DoHH2 Xenografts

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
Anti-Her2-smcc-DM1	600	11.9	3.3
Hu10F4v3-smcc-DM1	600	11.8	3.35
Hu10F4v3-smcc-DM1	300	5.9	3.35
Anti-Her2-MC-MMAF	600	9.9	4.0
Hu10F4v3-MC-MMAF	600	13.1	3.04
Hu10F4v3-MC-MMAF	300	6.6	3.04
Naked hu10F4v3	--	13.1	--

Table 13C
In Vivo Tumor Volume Reduction,
Hu10F4v3 MMAF and DM1 Conjugate Administration
In Granta-519 Xenografts

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
Anti-Her2-smcc-DM1	300	5.9	3.3
Hu10F4v3-smcc-DM1	300	5.9	3.35
Hu10F4v3-smcc-DM1	150	2.9	3.35
Anti-Her2-MC-MMAF	300	4.9	4.0
Hu10F4v3-MC-MMAF	300	6.6	3.04
Hu10F4v3-MC-MMAF	150	3.3	3.04
Naked hu10F4v3	--	6.6	--

Anti-CD22 hu10F4v3 –smcc-DM1 and -MC-MMAF ADCs showed potent tumor reduction in all of the xenograft models tested.

Example 10: Preparation of Cysteine Engineered Anti-CD22 Antibodies

Preparation of cysteine engineered anti-CD22 antibodies was performed as disclosed herein. DNA encoding the 10F4v3 antibody, having the same variable and constant region sequences as 10F4v2 (light chain, SEQ ID NO:87; and heavy chain, SEQ ID NO:88, Figure 5B), was mutagenized by methods disclosed herein to modify the light chain, the heavy chain or the Fc region of the heavy chain. DNA encoding the light chain was mutagenized to substitute cysteine for valine at Kabat position 205 in the light chain (sequential position 210) as shown in Figure 17A (light chain SEQ ID NO:91 of humanized antibody 10F4v3 thiomab). DNA encoding the heavy chain was mutagenized to substitute cysteine for alanine at EU position 118 in the heavy chain (sequential position 121) as shown in Figure 17B (heavy chain SEQ ID NO:92 of humanized antibody 10F4v3 thiomab). The Fc region was mutagenized to substitute cysteine for serine at EU position 400 in the heavy chain Fc region (sequential position 403) as shown in Figure 17C (heavy chain SEQ ID NO:93).

Preparation of cysteine engineered anti-CD22 antibodies for conjugation by reduction and reoxidation.

Full length, cysteine engineered anti-CD22 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 thiol concentration by

reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm.

Example 11: Preparation of cysteine engineered anti-CD22 antibody drug conjugates by conjugation of cysteine engineered anti-CD22 antibodies and drug-linker intermediates.

After the reduction and reoxidation procedures of Example 10, the cysteine engineered anti-CD22 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-CD22 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.

Preparation of hu 10F4v3 HC(A118C) thiomab-BMPEO-DM1 was performed as follows. The free cysteine on hu 10F4v3 HC(A118C) thiomab was modified by the bis-maleimido reagent BM(PEO)4 (Pierce Chemical), leaving an unreacted maleimido group on the surface of the antibody. This was accomplished by dissolving BM(PEO)4 in a 50% ethanol/water mixture to a concentration of 10 mM and adding a tenfold molar excess of BM(PEO)4 to a solution containing hu4D5Fabv8-(V110C) ThioFab in phosphate buffered saline at a concentration of approximately 1.6 mg/ml (10 micromolar) and allowing it to react for 1 hour. Excess BM(PEO)4 was removed by gel filtration (HiTrap column, Pharmacia) in 30 mM citrate, pH 6 with 150 mM NaCl buffer. An approximate 10 fold molar excess DM1 dissolved in dimethyl acetamide (DMA) was added to the hu4D5Fabv8-(V110C) ThioFab-BMPEO intermediate. Dimethylformamide (DMF) may also be employed to dissolve the drug moiety reagent. The reaction mixture was allowed to react overnight before gel filtration or dialysis into PBS to remove unreacted drug. Gel filtration

on S200 columns in PBS was used to remove high molecular weight aggregates and furnish purified hu 10F4v3 HC(A118C) thiomab-BMPEO-DM1.

By the same protocols, control HC (A118C) MAb-MC-MMAF, control HC ThioMAb-MC-MMAF, contol HCThioMAb-MCvcPAB-MMAE, and control HC ThioMab-BMPEO-DM1 were prepared.

By the procedures above, the following cysteine engineered anti-CD22 antibody drug conjugates were prepared and tested:

thio hu thio-HC-10F4v3-MC-MMAF by conjugation of A118C thio hu 10F4v3 and MC-MMAF;

thio hu thio-HC-10F4v3-MC-val-cit-PAB-MMAE by conjugation of A118C thio hu 10F4v3 and MC-val-cit-PAB-MMAE;

thio hu HC-10F4v3-bmpeo-DM1 by conjugation of A118C thio hu HC-10F4v3 and bmpeo-DM1;

thio hu LC-10F4v3-MC-val-cit-PAB-MMAE by conjugation of V205C thio hu LC-10F4v3 and MC-val-cit-PAB-MMAE; and

thio hu Fc-10F4v3-MC-val-cit-PAB-MMAE by conjugation of S400C thio hu Fc-10F4v3 and MC-val-cit-PAB-MMAE.

Example 12: Characterization of Binding Affinity of Cysteine Engineered ThioMAb Drug Conjugates to Cell Surface Antigen

The binding affinity of thio hu 10F4v3 drug conjugates to CD22 expressed on BJAB-luc cells was determined by FACS analysis. Briefly, approximately 1x10^6 cells in 100 µl were contacted with varying amounts of one of the following anti-CD22 ThioMAb drug conjugates: thio hu LC(V205C) 10F4v3-MCvcPAB-MMAE, thio hu Fc(S400C) 10F4v3-MCvcPAB-MMAE, thio hu HC(A118C) 10F4v3-MCvcPAB-MMAE, thio hu HC(A118C) 10F4v3-MC-MMAF, or thio hu HC(A118C) 10F4v3-BMPEO-DM1 (see Figures 18A-18E, respectively). Anti-CD22 antibody bound to the cell surface was detected using biotinylated goat anti-huFc plus Streptavidin-PE. The plots of Figures 18A-18E

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indicate that antigen binding was approximately the same for all of the thiomab drug conjugates tested.

Example 13: Assay for In Vivo Tumor Volume Reduction by Anti-CD22 ThioMab Drug Conjugates

The ability of the thiomab drug conjugates prepared according to Example 11 to reduce B-cell tumor volume in xenograft models was tested according to the procedure disclosed in Example 9, herein. To SCID mice having Granta-519 cell xenograft tumors, contol and anti-CD22 humanized 10F4v3 thiomab drug conjugates were administered at Day 0 in the doses shown in Table 14, below. The control HC(A118C) thiomab was anti-HER2 4D5 antibody.

Table 14
In Vivo Tumor Volume Reduction,
Thio Hu10F4v3 MMAE and MMAF Conjugate Administration
In Granta-519 Xenografts

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
Thio Control HC(A118C)-MC-MMAF	100	3.99	1.65
Thio Control HC(A118C)-MCvcPAB-MMAE	100	4.33	1.55
Thio 10F4v3-HC(A118C)-MC-MMAF	100	3.41	1.95
Thio 10F4v3-LC(V205C)-MCvcPAB-MMAE	100	4.23	1.6
Thio 10F4v3-HC(A118C)-MCvcPAB-MMAE	100	3.76	1.8
Thio 10F4v3-Fc(S400C)-MCvcPAB-MMAE	100	4.23	1.6

The results of this experiment are shown in Figure 19. Administration of the thio 10F4v3-LC-(V205C)-MCvcPAB-MMAE and thio 10F4v3-HC(A118C)-MCvcPAB-MMAE thiomab drug conjugates at the doses shown in Table 14 caused a reduction in mean tumor volume for the duration of the study.

Additional thiomab drug conjugates were tested in Granta-519 xenografts in CB17 SCID mice using the same protocol, although different drug doses were tested. The control

antibody or control thiomab was anti-HER2 4D5 antibody or HC(A118C) thiomab. The results are shown in Table 15, below.

Table 15
In Vivo Tumor Volume Reduction,
Thio Hu10F4v3 MMAE, MMAF, and DM1 Conjugate Administration
In Granta-519 Xenografts

Antibody administered	Dose MMAF or DM1 ($\mu g/m^2$)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
10F4v3-MC-MMAF	150	3.2	3.1
Thio Control HC(A118C)-BMPEO-DM1	300	10.3	1.9
Thio 10F4v3-HC(A118C)-BMPEO-DM1	150	5.2	1.9
Thio 10F4v3-HC(A118C)-BMPEO-DM1	300	10.4	1.9
Thio Control HC(A118C)-MCvcPAB-MMAE	150	6.5	1.55
Thio 10F4v3-HC(A118C)-MCvcPAB-MMAE	150	5.3	1.9
Thio 10F4v3-HC(A118C)-MCvcPAB-MMAE	75	2.7	1.9
Thio Control HC(A118C)-MC-MMAF	150	5.2	1.9
Thio 10F4v3-HC(A118C)-MC-MMAF	150	5.1	1.95
Thio 10F4v3-HC(A118C)-MC-MMAF	75	2.6	1.95

The results of this experiment are shown in Figure 20A. Administration of the thio 10F4v3-HC(A118C)-MCvcPAB-MMAE thiomab drug conjugate at 150 and 75 $\mu g/m^2$ caused a reduction in mean tumor volume for the duration of the study. In the same study, the percent body weight change in the first 7 days was determined in each dosage group. The results plotted in Figure 20B indicate administration of these thiomab drug conjugates did not cause weight loss during this time.

In a similar study, using the same xenograft study protocol as disclosed in the above examples, varying the TDCs and doses administered, the efficacy of TDCs in follicular lymphoma DOHH2 xenografts in CB17 SCID mice was studied. The TDCs and doses are shown in Table 16, below.

Table 16
In Vivo Tumor Volume Reduction,
Thio Hu10F4v3 MMAE, MMAF, and DM1 Conjugate Administration
In DOHH2 Xenografts

Antibody administered	Dose MMAF or DM1 (μg/m^2)	Dose Ab (mg/kg)	Drug ratio (Drug /Ab)
10F4v3-MC-MMAF	300	6.4	3.1
Thio Control HC(A118C)-BMPEO-DM1	600	21.9	1.79
Thio 10F4v3-HC(A118C)-BMPEO-DM1	600	20.8	1.9
Thio 10F4v3-HC(A118C)-BMPEO-DM1	300	10.4	1.9
Thio Control HC(A118C)-MCvcPAB-MMAE	600	26.0	1.55
Thio 10F4v3-HC(A118C)-MCvcPAB-MMAE	600	21.4	1.9
Thio 10F4v3-HC(A118C)-MCvcPAB-MMAE	300	10.7	1.9
Thio Control HC(A118C)-MC-MMAF	600	20.8	1.9
Thio 10F4v3-HC(A118C)-MC-MMAF	600	20.4	1.95
Thio 10F4v3-HC(A118C)-MC-MMAF	300	10.2	1.95

Figure 20C is a graph plotting changes in mean tumor volume over time in the follicular lymphoma DOHH2 xenograft in CB17 SCID mice treated with the same heavy chain A118C anti-CD22 TDCs, but at higher doses as shown in Table 16. The anti-CD22 10F4v3-HC(A118C)-MCvcPAB-MMAE TDC appeared to be the most efficacious of the test agents in this study. However, at the increased dose levels in this experiment, some efficacy was noted in the anti-HER2-HC(A118C)-MCvcPAB-MMAE controls. This activity is possibly attributable to release of the drug from the ADC in circulation. The anti-CD22 hu10F4-HC(A118C)-MC-MMAF and –BMPEO-DM1 test agents showed intermediate efficacy and, consistent with the increased stability of these linkers, the non-binding anti-HER2 controls showed little activity. Figure 20D is a plot of percent weight change in the mice from the DOHH2 xenograft study showing that there was no significant change in weight during the first 14 days of the study.

Example 14: Safety of Anti-CD22 Drug Conjugates in Rats and Cynomolgus Monkeys

The hu10F4 anti-CD22 antibody cross-reacts with cynomolgus (cyno) monkey CD22 with an affinity equivalent to human CD22. The hu10F4 anti-CD22 antibody does not cross-react with rat CD22. As a result, the target-independent and target-dependent safety and toxicity of anti-CD22 drug conjugates were assessed in rat and cyno, respectively.

Safety and toxicity in rats.

For safety and toxicity studies in rats, two studies were performed. In one study, rats were dosed intravenously on Day 1 with hu10F4v3-SMCC-DM1, -SPP-DM1, -MC-vc-PAB-MMAE, or –MC-MMAF conjugates in which the drug was linked via a cleavable (-vc- or –spp-) or uncleavable (MC or SMCC (also referred to as MCC)) linker. Vehicle was administered as the control. Blood samples were collected on Day 5 for pharmacokinetic analysis, and on Day 12 (at necropsy). Clinical observations and body weight recordings were conducted at least three times per week. Serum AST (aspartate aminotransferase) was monitored as an indication of toxicity. Serum AST levels were increased at Day 5 relative to Day 0 in rats dosed with 20 mg/kg hu10F4v3-vcMMAE and hu10F4v3-SPP-DM1 comprising cleavable linkers (Figure 21A). Neutrophil levels were increased at Day 5 relative to Day 0 in rats dosed with 20 mg/kg hu10F4v3-MC-MMAF or hu10F4v3-MCC-DM1 (uncleavable linkers, Figure 21B). Neutrophil levels were decreased at Day 5 relative to Day 0 in rats dosed with hu10F4v3-vc-MMAE or hu10F4v3-SPP-DM1. Increased serum AST and decreased neutrophils in rats dosed with ADCs comprising cleavable linkers indicates increased toxicity of such ADCs

In the same rat study, six animals per group were dosed with 20, 40, or 60 mg/kg hu10F4v3-MC-MMAF or hu10F4v3-SMCC-DM1 at Day 1 and monitored for twelve days. In animals dosed with hu10F4v3-MC-MMAF, there were no observations in the following indicators: decreased body weight, increases in serum liver enzymes, decreases in platelets, or decreases in neutrophils. In rats dosed with hu10F4v3-SMCC-DM1, reversible decreased body weight and reversible increases in serum liver enzymes were observed at dose levels of 40 and 60 mg/kg, whereas reversible decreases in neutrophils and transient decreases in platelets were observed at 60 mg/kg doses.

Safety and toxicity in cynomolgus monkeys.

To assess safety and toxicity of anti-CD22 ADCs in a primate model, thirty cyno monkeys were assigned to the following treatment groups: vehicle control (6 animals), hu10F4v3-SMCC-DM1 at doses of 2, 4, and 6 mg/m^2 drug dose (equivalent to 0, 10, 20, and 30 mg/kg antibody dose; 4 animals per dosage group), and hu10F4v3-MC-MMAF at doses of 2, 4, and 6 mg/m^2 (4 animals per dosage group). Animals were dosed intravenously on Day 1 and Day 22. The animals were evaluated for changes in body weight, food consumption, and pathology indices. Blood samples were collected and assayed to assess toxicological, pharmacodynamic, and anti-drug antibody effects. One-half of the animals in each group were euthanized at each of Day 25 and Day 43 and tissue samples were collected.

No noticeable body weight changes were noted in either ADC group. Levels of serum liver enzymes AST (aspartate aminotransferase), ALT (aminotransferase) and GGT (gamma-glutamyltranspeptidase) were assayed according to standard methods well known in the relevant arts. Reversible increases in serum liver enzymes were observed in animals dosed at 30 mg/kg with either ADC, although ALT was elevated in the DM1 group, whereas AST and GGT were elevated in the MMAF group. Sciatic nerve degeneration was minimal to mild in the DM1 group in 2 of 4 animals at a dose of 20 mg/kg and in 4 of 4 animals at a dose of 30 mg/kg. Sciatic nerve degeneration was minimal in the MMAF group in 1 of 4 animals at a dose of 30 mg/kg. Tissue from various organs was examined microscopically. Two of four animals in the 30 mg/kg MMAF group had lung lesionsof unknown significance, whereas none were observed in the DM1 group.

Depletion of peripheral B cells by the hu10F4v3-MC-MMAF and –SMCC-DM1 ADCs was determined by measuring $CD20^+$ cell levels in blood over 43 days in cyno monkeys dosed at Day 0 and Day 22. Blood collected periodically during the study was assayed by FACS using a fluorescently labeled anti-CD20 antibody. The anti-CD22 MMAF and DM1 ADCs deplete cyno peripheral B cells as shown in Figure 22A (MMAF group) and Figure 22B (DM1 group). No significant effects of MMAF or DM1 ADCs were observed for other lymphocyte populations as shown in Figures 23A and 23B in which it is shown that $CD4^+$ cells were not significantly depleted over the same time period.

Hu10F4v3-SMCC-DM1 depleted germinal center B cells in the cyno monkey tonsil samples relative to control as shown in the photomicrographs in Figures 24A and 24B. Exemplary germinal centers are circled in Figure 24A. Complete ablation of germinal center B cells was observed at the 10 mg/kg dose level as shown if Figure 24B. The same results were obtained following administration of the hu10F4v3-MC-MMAF ADC under the same conditions.

Hu10F4v3-MC-MMAF dosed at 10 mg/kg depleted dividing B cells from the spleen follicle germinal centers of cyno monkeys. See the diagram in Figure 25A and the tissue photomicrographs in Figures 25B and 25C. The same results were obtained when the hu10F4v3-SMCC-DM1 ADC was tested under the same conditions. Germinal centers appear as dark regions in Figure 25B using Ki-67 stain and as unstained areas surrounded by dark regions when stained with detectably labeled anti-IgD in Figure 25D. Loss of the germinal centers due to depletion of germinal center B cells by anti-10F4v3-MC-MMAF is shown in Figures 25C and 25E. Thus, these anti-mitotic drugs have an impact on proliferating B cell populations.

The following hybridoma has been deposited with the American Type Culture Collection, PO Box 1549, Manassas, VA, 20108, USA (ATCC):

Cell Lines	ATCC Accession No.	Deposit Date
Hybridoma 10F4.4.1	PTA-7621	May 26, 2006
Hybridoma 5E8.1.8	PTA-7620	May 26, 2006

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable deposit for 30 years from the date of deposit. These cell lines will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the cell lines to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the cell lines to one determined by the U.S. Commissioner of Patents and

Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if the deposited cell lines should be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a specimen of the same cell line. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

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.

WHAT IS CLAIMED IS:

1. An antibody that binds to CD22, wherein the antibody comprises (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:9, 10, 19-23, 32 and 33, 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:2;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;
(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14.
2. The antibody of claim 1, comprising an HVR-L1 comprising an amino acid sequence that conforms to the consensus sequence of SEQ ID NO:10.
3. The antibody of claim 2, further comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.
4. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:9, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.
5. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:19, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.
6. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:20, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEEQ ID NO:6.
7. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:21, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

8. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:22, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

9. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:23, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

10. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:32, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

11. The antibody of claim 1, wherein the HVR-L1 comprises SEQ ID NO:33, and the antibody further comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4; and an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6.

12. The antibody of any of claim 1, further comprising at least one framework selected from a VH subgroup III consensus framework and a VL subgroup I consensus framework.

13. The antibody of claim 1, wherein the antibody comprises a heavy chain variable domain having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO:16.

14. The antibody of claim 1, wherein the antibody comprises a light chain variable domain having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO:17.

15. The antibody of claim 1, wherein the antibody comprises a light chain variable domain having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO:18.

16. The antibody of claim 1, wherein the antibody comprises a heavy chain variable domain comprising one, two, three or four framework amino acid sequences selected from SEQ ID NOs:1, 3, 5, and 7.

17. The antibody of claim 1, wherein the antibody comprises a light chain variable domain comprising one, two, three or four framework amino acid sequences selected from SEQ ID NOs:8, 11, 13 and 15.

18. The antibody of claim 13, further comprising a light chain variable domain having at least 90% sequence identity to animo acid sequence selected from SEQ ID NO:17.
19. The antibody of claim 13, further comprising a light chain variable domain having at least 90% sequence identity to animo acid sequence selected from SEQ ID NO:18.
20. The antibody of claim 1, wherein the antibody comprises a heavy chain having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO:88.
21. The antibody of claim 1, wherein the antibody comprises a light chain having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NO:87.
22. The antibody of claim 1, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:88 and a light chain having an amino acid sequence of SEQ ID NO:87.
23. An antibody that binds to CD22, wherein the antibody comprises a heavy chain variable domain having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:16.
24. The antibody of claim 23, further comprising a light chain variable domain having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:17.
25. The antibody of claim 23, further comprising a light chain variable domain having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:18.
26. The antibody of claim 1, wherein the antibody comprises one, two, three, four, five or six of the HVRs from the antibody produced by the hybridoma ATCC accession no. PTA-7621 (10F4.4.1).
27. An antibody comprising one, two, three, four, five or six of the HVRs from the antibody produced by the hybridoma ATCC accession no. PTA-7620 (5E8.1.8).
28. The antibody of claim 1, wherein the antibody is humanized.
29. The antibody of claim 23, wherein the antibody is humanized.
30. The antibody of claim 1, wherein the CD22 is mammalian CD22.
31. The antibody of claim 30, wherein the CD22 is selected from rodent CD22 and primate CD22.
32. The antibody of claim 31, wherein the CD22 is human CD22.
33. The antibody of claim 23, wherein the CD22 is mammalian CD22.
34. The antibody of claim 33, wherein the CD22 is selected from rodent CD22 and primate CD22.
35. The antibody of claim 34, wherein the CD22 is human CD22.

36. A polynucleotide encoding an antibody of claim 1.
37. A polynucleotide encoding an antibody of claim 23.
38. A vector comprising the polynucleotide of claim 36.
39. A vector comprising the polynucleotide of claim 37.
40. A host cell comprising the vector of claim 38.
41. A host cell comprising the vector of claim 39.
42. The host cell of claim 40, wherein the host cell is eukaryotic.
43. The host cell of claim 42, wherein the host cell is a CHO cell.
44. The host cell of claim 41, wherein the host cell is eukaryotic.
45. The host cell of claim 44, wherein the host cell is a CHO cell.

46. A method of making an anti-CD22 antibody, wherein the method comprises a) culturing the host cell of claim 38 under conditions suitable for expression of the polynucleotide encoding the antibody, and b) isolating the antibody.
47. A method of making an anti-CD22 antibody, wherein the method comprises a) culturing the host cell of claim 39 under conditions suitable for expression of the polynucleotide encoding the antibody, and b) isolating the antibody.
48. The antibody of claim 28, wherein the CD22 is expressed on the surface of a cell.
49. The antibody of claim 48, wherein the cell is a B cell.
50. The antibody of claim 29, wherein the CD22 is expressed on the surface of a cell.
51. The antibody of claim 50, wherein the CD22 is a B cell.
52. The antibody of claim 1, wherein the antibody binds to an epitope within a region of CD22 from amino acid 22-240 of SEQ ID NO:27.
53. The antibody of claim 23, wherein the antibody binds to an epitope within a region of CD22 from amino acid 22-240 of SEQ ID NO:27.
54. The antibody of claim 49, wherein the B cell is associated with a B cell proliferative disorder.
55. The antibody of claim 54, wherein the B cell proliferative disorder is a cancer.
56. The antibody of claim 54, wherein the B cell proliferative disorder is selected from - 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.

57. The antibody of claim 51, wherein the B cell is associated with a B cell proliferative disorder.

58. The antibody of claim 57, wherein the B cell proliferative disorder is a cancer.

59. The antibody of claim 57, wherein the B cell proliferative disorder is selected from - 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.The antibody of any of claims 1-14, 16-23, or 30-33, wherein the antibody is a monoclonal antibody.

60. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

61. The antibody of claim 60, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

62. The antibody of claim 60, wherein the antibody is humanized.

63. The antibody of claim 60, wherein the antibody is human.

64. The antibody of claim 23, wherein the antibody is a monoclonal antibody.

65. The antibody of claim 64, wherein the antibody is an antibody fragment selected from a Fab, Fab'-SH, Fv, scFv, or $(Fab')_2$ fragment.

66. The antibody of claim 64, wherein the antibody is humanized.

67. The antibody of claim 64, wherein the antibody is human.

68. The antibody of claim 1, wherein the antibody binds to the same epitope as an antibody selected from ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); and an antibody comprising a heavy chain sequence of SEQ ID NO:88 and a light chain sequence of SEQ ID NO:87.

69. The antibody of claim 23, wherein the antibody binds to the same epitope as an antibody selected from ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); and an antibody comprising a heavy chain sequence of SEQ ID NO:88 and a light chain sequence of SEQ ID NO:87.

70. A method of detecting the presence of CD22 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 CD22, and detecting whether a complex is formed between the antibody and CD22.

71. The method of claim 70, wherein the biological sample is from a patient suspected of having a B cell proliferative disorder.

72. The method of claim 71, wherein the B cell proliferative disorder is selected from - 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.

73. A method of detecting the presence of CD22 in a biological sample, the method comprising contacting the biological sample with an antibody of claim 23 under conditions permissive for binding of the antibody to CD22, and detecting whether a complex is formed between the antibody and CD22.

74. The method of claim 73, wherein the biological sample is from a patient suspected of having a B cell proliferative disorder.

75. The method of claim 74, wherein the B cell proliferative disorder is selected from - 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.

76. An immunoconjugate comprising an antibody of claim 1 covalently attached to a cytotoxic agent.

77. An immunoconjugate comprising an antibody of claim 23 covalently attached to a cytotoxic agent.

78. The immunoconjugate of claim 76, wherein the cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

79. The immunoconjugate of claim 78, the immunoconjugate having the formula Ab-(L-D)p, wherein:

(a) Ab is the antibody of claim 1;

(b) L is a linker;

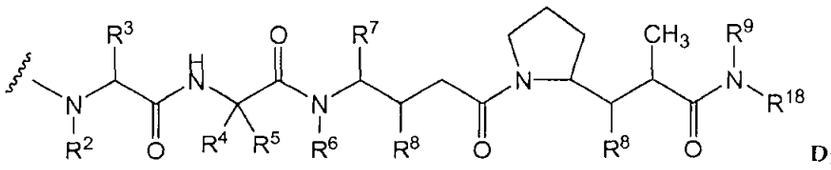
(c) D is a drug moiety.

80. The immunoconjugate of claim 79, 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

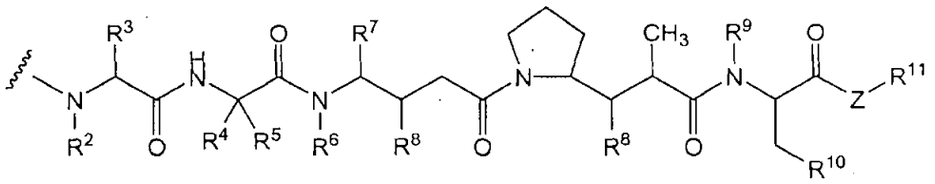
(SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

81. The immunoconjugate of claim 79, wherein D is selected from an auristatin and dolostantin.

82. The immunoconjugate of claim 81, 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.

83. The immunoconjugate of claim 76, having in vitro or in vivo cell killing activity.

84. The immunoconjugate of claim 79, wherein the linker is attached to the antibody through a thiol group on the antibody.

85. The immunoconjugate of claim 79, wherein the linker is cleavable by a protease.

86. The immunoconjugate of claim 80, wherein the linker comprises a val-cit dipeptide.

87. The immunoconjugate of claim 79, wherein the linker comprises a p-aminobenzyl unit.

88. The immunoconjugate of claim 80, wherein the linker comprises 6-maleimidocaproyl.

89. The immunoconjugate of claim 82, wherein the drug is selected from MMAE and MMAF.

90. The immunoconjugate of claim 89, wherein the drug is MMAE.

91. The immunoconjugate of claim 89, wherein the drug is MMAF.

92. The immunoconjugate of claim 77, wherein the cytotoxic agent is selected from a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

93. The immunoconjugate of claim 92, the immunoconjugate having the formula Ab-(L-D)p, wherein:

(a) Ab is the antibody of claim 23;

(b) L is a linker;

(c) D is a drug moiety.

94. The immunoconjugate of claim 93, 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), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

95. The immunoconjugate of claim 93, wherein the linker, L, is cleavable by a protease.

96. The immunoconjugate of claim 94, wherein L comprises a val-cit dipeptide.

97. The immunoconjugate of claim 93, wherein L comprises a p-aminobenzyl unit.

98. The immunoconjugate of claim 97, wherein the p-aminobenzyl unit is p-aminobenzyloxycarbonyl (PAB).

99. The immunoconjugate of claim 94, wherein L comprises 6-maleimidocaproyl (MC).

100. The immunoconjugate of claim 94, wherein the linker comprises 6-maleimidocaproyl and p-aminobenzyloxycarbonyl.

101. The immunoconjugate of claim 79, wherein the immunoconjugate has the formula Ab-(L-MMAE)p,

wherein L is a linker and p ranges from 2 to 5.

102. The immunoconjugate of claim 101, wherein L comprises val-cit.

103. The immunoconjugate of claim 101, wherein L comprises MC.

104. The immunoconjugate of claim 101, wherein L comprises PAB.

105. The immunoconjugate of claim 101, wherein L comprises MC-PAB.

106. The immunoconjugate of claim 93, wherein the immunoconjugate has the formula Ab-(L-MMAE)p,

wherein L is a linker and p ranges from 2 to 5.

107. The immunoconjugate of claim 106, wherein L comprises val-cit.

108. The immunoconjugate of claim 106, wherein L comprises MC.

109. The immunoconjugate of claim 106, wherein L comprises PAB.

110. The immunoconjugate of claim 106, wherein L comprises MC-PAB.

111. The immunoconjugate of claim 79, wherein the immunoconjugate has the formula Ab-(L-MMAF)p,
wherein L is a linker and p ranges from 2 to 5.

112. The immunoconjugate of claim 111, wherein L comprises val-cit.

113. The immunoconjugate of claim 111, wherein L comprises MC.

114. The immunoconjugate of claim 111, wherein L comprises PAB.

115. The immunoconjugate of claim 111, wherein L comprises MC-PAB.

116. The immunoconjugate of claim 93, wherein the immunoconjugate has the formula Ab-(L-MMAF)p,
wherein L is a linker and p ranges from 2 to 5.

117. The immunoconjugate of claim 116, wherein L comprises val-cit.

118. The immunoconjugate of claim 116, wherein L comprises MC.

119. The immunoconjugate of claim 116, wherein L comprises PAB.

120. The immunoconjugate of claim 116, wherein L comprises MC-PAB.

121. The immunoconjugate of claim 79, wherein D is a maytansinoid.

122. The immunoconjugate of claim 121, wherein D is selected from DM1, DM3, and DM4.

123. The immunoconjugate of claim 121, having in vitro or in vivo cell killing activity.

124. The immunoconjugate of claim 121, wherein the linker is attached to the antibody through a thiol group on the antibody.

125. The immunoconjugate of claim 121, 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).

126. The immunoconjugate of claim 122, wherein the drug is DM1.

127. The immunoconjugate of claim 122, wherein L comprises a SPP.

128. The immunoconjugate of claim 122, wherein L comprises SMCC.

129. The immunoconjugate of claim 122, wherein p is from 2-4.

130. The immunoconjugate of claim 122, wherein p is from 3-4.

131. The immunoconjugate of claim 93, wherein D is a maytansinoid.

132. The immunoconjugate of claim 131, wherein D is selected from DM1, DM3, and DM4.

133. The immunoconjugate of claim 131, having in vitro or in vivo cell killing activity.

134. The immunoconjugate of claim 131, wherein the linker is attached to the antibody through a thiol group on the antibody.

135. The immunoconjugate of claim 131, 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).

136. The immunoconjugate of claim 132, wherein the drug is DM1.

137. The immunoconjugate of claim 136, wherein L comprises a SPP.

138. The immunoconjugate of claim 136, wherein L comprises SMCC.

139. The immunoconjugate of claim 136, wherein p is from 2-4.

140. The immunoconjugate of claim 136, wherein p is from 3-4.

141. A pharmaceutical composition comprising the immunoconjugate of claim 79 and a pharmaceutically acceptable carrier.

142. A method of treating a B cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 141.

143. The method of claim 142, wherein the B cell proliferative disorder is selected from - 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.

144. A pharmaceutical composition comprising the immunoconjugate of claim 93 and a pharmaceutically acceptable carrier.

145. A method of treating a B cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 144.

146. The method of claim 145, wherein the B cell proliferative disorder is selected from - 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.

147. A pharmaceutical composition comprising the immunoconjugate of claim 76, 77, 78, or 92 and a pharmaceutically acceptable carrier.

148. A method of treating a B cell proliferative disorder comprising administering to an individual an effective amount of the pharmaceutical composition of claim 147.

149. The method of claim 148, wherein the B cell proliferative disorder is selected from 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.

150. A method of inhibiting B cell proliferation comprising exposing a cell to an immunoconjugate of claim 79 under conditions permissive for binding of the immunoconjugate to CD22.

151. The method of claim 150, wherein the B cell proliferation is associated with a disorder selected from 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..

152. The method of claim 150, wherein the B cell is a xenograft.

153. The method of claim 150, wherein the exposing takes place in vitro.

154. The method of claim 150, wherein the exposing takes place in vivo.

155. 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.

156. The cysteine engineered antibody of claim 155, wherein the cysteine engineered antibody is more reactive than the parent antibody with a thio-reactive reagent.

157. The cysteine engineered antibody of claim 155 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.

158. The cysteine engineered antibody of claim 155 wherein the one or more free cysteine amino acid residues are located in a light chain.

159. The cysteine engineered antibody of claim 155, wherein the antibody is an immunoconjugate comprising the cysteine engineered antibody covalently attached to a cytotoxic agent.

160. The cysteine engineered antibody of claim 159, wherein the cytotoxic agent is selected fom a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

161. The cysteine engineered antibody of claim 155 wherein the antibody is covalently attached to a capture label, a detection label, or a solid support.

162. The cysteine engineered antibody of claim 161 wherein the antibody is covalently attached to a biotin capture label.

163. The cysteine engineered antibody of claim 161 wherein the antibody is covalently attached to a fluorescent dye detection label.

164. The cysteine engineered antibody of claim 163 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.

165. The cysteine engineered antibody of claim 161 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.

166. The cysteine engineered antibody of claim 161 wherein the antibody is covalently attached to a detection label by a chelating ligand.

167. The cysteine engineered antibody of claim 166 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

168. The antibody of claim 23 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.

169. The cysteine engineered antibody of claim 168, wherein the cysteine engineered antibody is more reactive than the parent antibody with a thio-reactive reagent.

170. The cysteine engineered antibody of claim 168 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.

171. The cysteine engineered antibody of claim 168 wherein the one or more free cysteine amino acid residues are located in a light chain.

172. The cysteine engineered antibody of claim 168, wherein the antibody is an immunoconjugate comprising the cysteine engineered antibody covalently attached to a cytotoxic agent.

173. The cysteine engineered antibody of claim 172, wherein the cytotoxic agent is selected fom a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope, and a nucleolytic enzyme.

174. The cysteine engineered antibody of claim 168 wherein the antibody is covalently attached to a capture label, a detection label, or a solid support.

175. The cysteine engineered antibody of claim 174 wherein the antibody is covalently attached to a biotin capture label.

176. The cysteine engineered antibody of claim 174 wherein the antibody is covalently attached to a fluorescent dye detection label.

177. The cysteine engineered antibody of claim 176 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.

178. The cysteine engineered antibody of claim 174 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.

179. The cysteine engineered antibody of claim 178 wherein the antibody is covalently attached to a detection label by a chelating ligand.

180. The cysteine engineered antibody of claim 179 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

181. The immunoconjugate of claim 93, wherein the linker is attached to the antbody through a thiol group on the antibody.

182. The antibody of claim 1 comprising an albumin binding peptide.

183. The antibody of claim 182, wherein the albumin binding peptide is selected from SEQ ID NOs:42-46.

184. The antibody of claim 23 comprising an albumin binding peptide.

185. The antibody of claim 184, wherein the albumin binding peptide is selected from SEQ ID NOs:42-46.

186. The antibody claim 1 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.

187. The antibody of claim 186, wherein a cysteine is at position 205 of the light chain.

188. The antibody of claim 186, wherein a cysteine is at position 118 of the heavy chain.

189. The antibody of claim 186, wherein a cysteine is at position 400 of the heavy chain.

190. The antibody of claim 186 wherein the antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

191. The antibody of claim 186 which is an antibody fragment.

192. The antibody of claim 191 wherein the antibody fragment is a Fab fragment.

193. The antibody of claim 186 which is selected from a chimeric antibody, a human antibody, or a humanized antibody.

194. The antibody of claim 186 which is produced in bacteria.

195. The antibody of claim 186 which is produced in CHO cells.

196. A method of determining the presence of a CD22 protein in a sample suspected of containing said protein, said method comprising exposing said sample to an antibody of claim 186 and determining binding of said antibody to said CD22 protein in said sample, wherein binding of the antibody to said protein is indicative of the presence of said protein in said sample.

197. The method of Claim 196 wherein said sample comprises a cell suspected of expressing said CD22 protein.

198. The method of Claim 196 wherein said cell is B cell.

199. The method of Claim 196 wherein the antibody is covalently attached to a label selected from a fluorescent dye, a radioisotope, biotin, or a metal-complexing ligand.

200. A pharmaceutical formulation comprising the anti-CD22 antibody of claim 186, and a pharmaceutically acceptable diluent, carrier or excipient.

201. The antibody of claim 186 wherein the antibody is covalently attached to an auristatin or a maytansinoid drug moiety whereby an antibody drug conjugate is formed.

202. The antibody-drug conjugate of claim 201 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:

$$Ab-(L-D)_p \qquad \mathbf{I}$$

where p is 1, 2, 3, or 4.

203. The antibody-drug conjugate compound of claim 201 wherein p is 2.

204. The antibody-drug conjugate compound of claim 201 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;

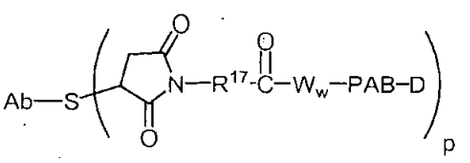
each W is independently an Amino Acid unit;

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.

205. The antibody-drug conjugate compound of claim 204 having the formula:



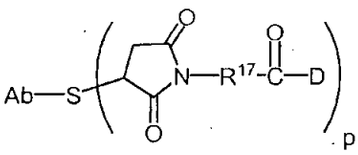
where PAB is para-aminobenzylcarbamoyl, and R^{17} is a divalent radical selected from $(CH_2)_r$, C_3-C_8 carbocyclyl, O–$(CH_2)_r$, arylene, $(CH_2)_r$–arylene, –arylene–$(CH_2)_r$–, $(CH_2)_r$–(C_3-C_8 carbocyclyl), (C_3-C_8 carbocyclyl)–$(CH_2)_r$, C_3-C_8 heterocyclyl, $(CH_2)_r$–(C_3-

C_8 heterocyclyl), $-(C_3\text{-}C_8$ 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, $C_1\text{-}C_6$ alkyl, phenyl, or benzyl; and r is independently an integer ranging from 1 to 10.

206. The antibody-drug conjugate compound of claim 204 wherein W_w is valine-citrulline.

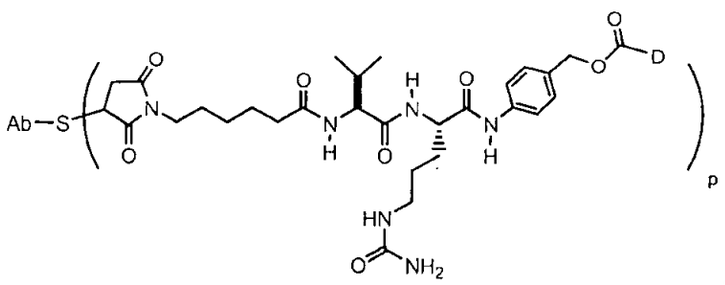
207. The antibody-drug conjugate compound of claim 204 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

208. The antibody-drug conjugate compound of claim 204 having the formula:



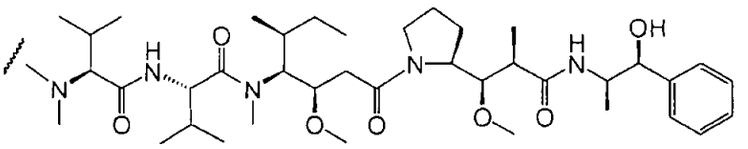
209. The antibody-drug conjugate compound of claim 208 wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

210. The antibody-drug conjugate compound of claim 204 having the formula:



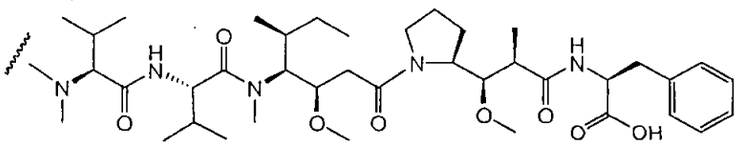
211. The antibody-drug conjugate compound of claim 202 wherein L is SMCC, SPP or BMPEO.

212. The antibody-drug conjugate compound of claim 202 wherein D is MMAE, having the structure:



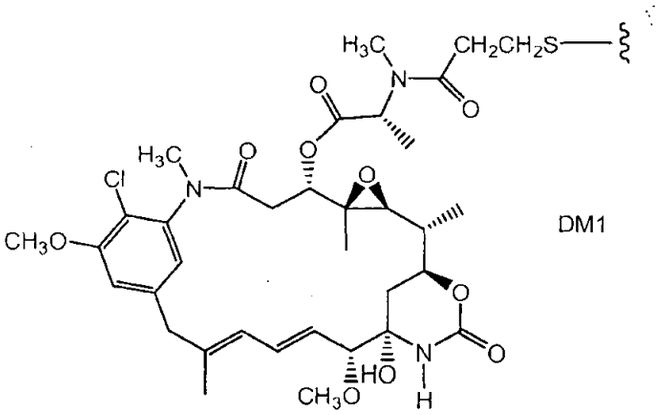
wherein the wavy line indicates the attachment site to the linker L.

213. The antibody-drug conjugate compound of claim 202 wherein D is MMAF, having the structure:



wherein the wavy line indicates the attachment site to the linker L.

214. The antibody-drug conjugate compound of claim 202 wherein D is DM1, having the structure:



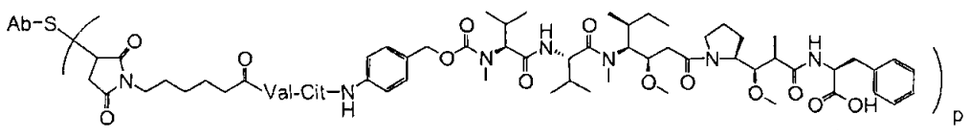
DM1

wherein the wavy line indicates the attachment site to the linker L.

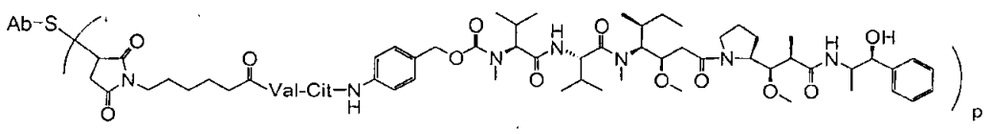
215. The antibody-drug conjugate compound of claim 201 wherein the parent anti-CD22 antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody, and an antibody fragment.

216. The antibody-drug conjugate compound of claim 201 wherein the antibody fragment is a Fab fragment.

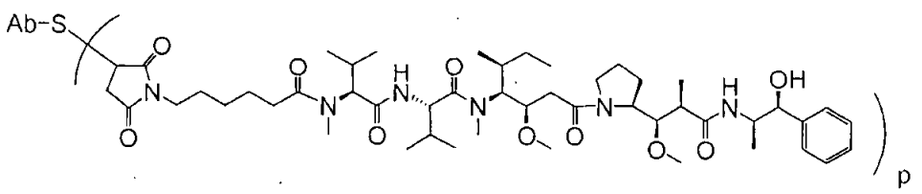
217. An antibody-drug conjugate compound selected from the structures:



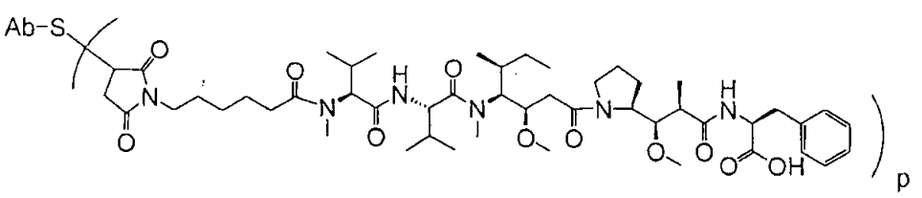
Ab-MC-vc-PAB-MMAF



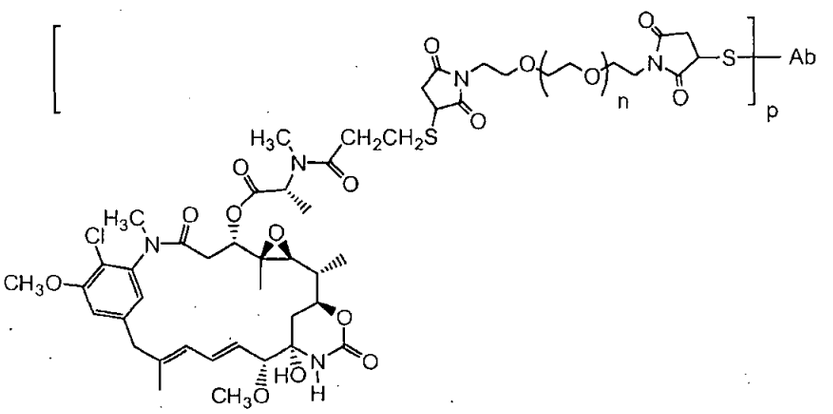
Ab-MC-vc-PAB-MMAE



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-CD22 antibody of claim 186.

218. The antibody drug conjugate of claim 201 wherein the auristatin is MMAE or MMAF.

219. The antibody drug conjugate of claim 202 wherein L is MC-val-cit-PAB or MC.

220. An assay for detecting B cells comprising:

(a) exposing cells to an antibody-drug conjugate compound of claim 199; and

(b) determining the extent of binding of the antibody-drug conjugate compound to the cells.

221. 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 201, whereby proliferation of the cancerous B cells is inhibited.

222. A pharmaceutical formulation comprising the antibody drug conjugate of claim 201, and a pharmaceutically acceptable diluent, carrier or excipient.

223. A method of treating cancer comprising administering to a patient the pharmaceutical formulation of claim 222.

224. The method of claim 223 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.

225. The method of claim 223 wherein the patient is administered a cytotoxic agent in combination with the antibody-drug conjugate compound.

226. An article of manufacture comprising

the pharmaceutical formulation of claim 220;

a container; and

a package insert or label indicating that the compound can be used to treat cancer characterized by the overexpression of a CD22 polypeptide.

227. The article of manufacture of claim 226 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.

228. A method for making an antibody drug conjugate compound comprising an anti-CD22 antibody (Ab) of claim 186, 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:

$$Ab-(L-D)_p \qquad I$$

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;

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.

229. The method of claim 228 further comprising the step of expressing the antibody in chinese hamster ovary (CHO) cells.

230. The method of claim 229 further comprising the step of treating the expressed antibody with a reducing agent.

231. The method of claim 230 wherein the reducing agent is selected from TCEP and DTT.

232. The method of claim 230 further comprising the step of treating the expressed antibody with an oxidizing agent, after treating with the reducing agent.

233. The method of claim 232 wherein the oxidizing agent is selected from copper sulfate, dehydroascorbic acid, and air.

234. The antibody of claim 186 wherein the antibody comprises a heavy chain sequence selected from any one of SEQ ID NOS:88, 92, or 93.

235. The antibody of claim 186 wherein the antibody comprises a light chain sequence selected from SEQ ID NOS:87 or 91.

236. The antibody of claim 186 wherein the antibody comprises a light chain sequence of SEQ ID NO:87 and a heavy chain sequence of SEQ ID NO:92.

237. The antibody of claim 186 wherein the antibody comprises a light chain sequence of SEQ ID NO:87 and a heavy chain sequence of SEQ ID NO:93.

238. The antibody of claim 186 wherein the antibody comprises a light chain sequence of SEQ ID NO:91 and a heavy chain sequence of SEQ ID NO:88.

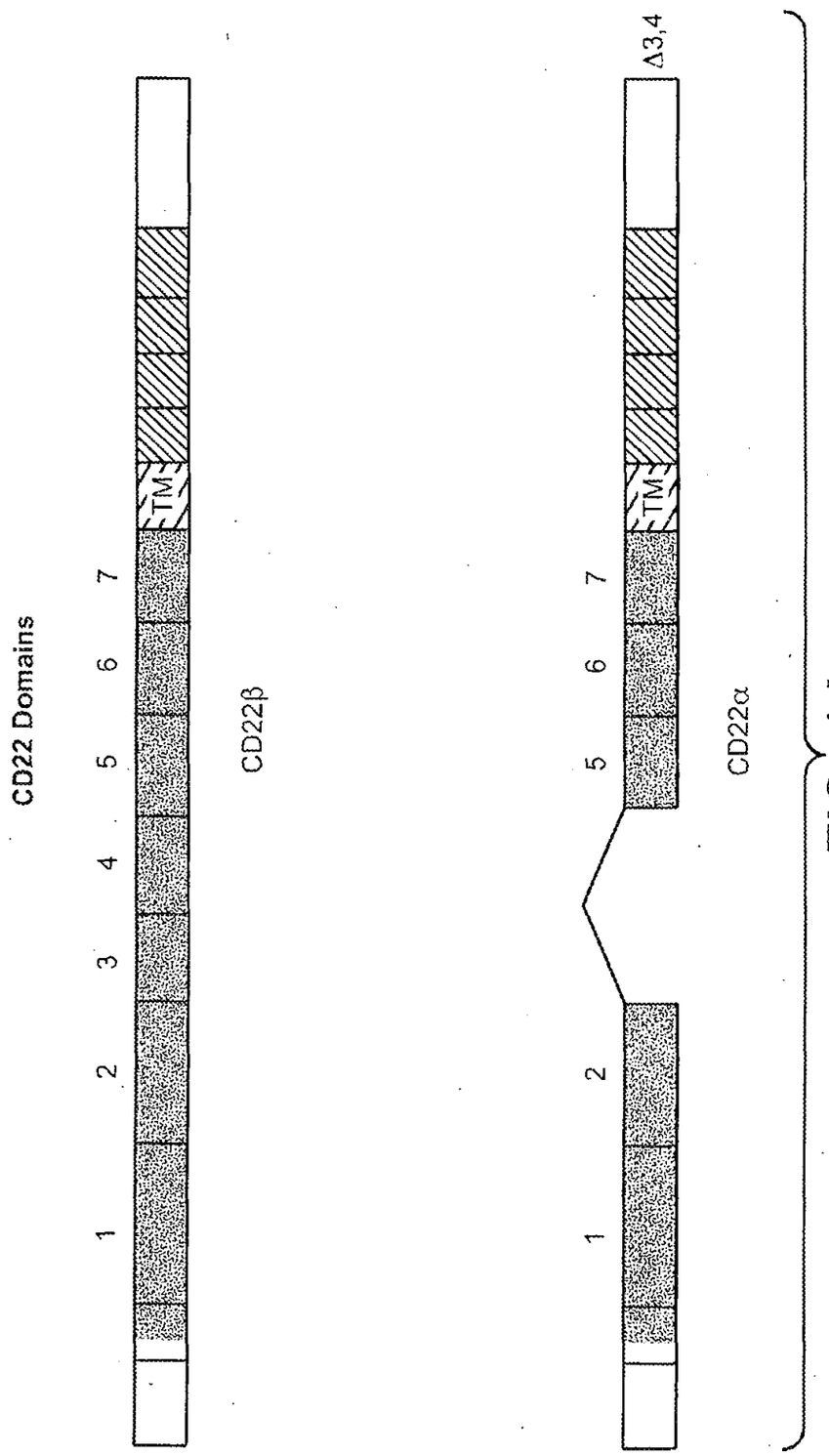


FIG. 1A

```
  1 mhllgpwlll lvleylafsd sskwvfehpe tlyawegacv wipctyrald gdlesfilfh
 61 npeynkntsk fdgtrlyest kdgkvpseqk rvqflgdknk nctlsihpvh lndsgqlglr
121 mesktekwme rihlnvserp fpphiqlppe iqesqevtlt cllnfscygy piqlqwlleq
181 vpmrqaavts tsltiksvft rselkfspqw shhgkivtcq lqdadgkfls ndtvqlnvkh
241 tpkleikvtp sdaivregds vtmtcevsss npeyttvswl kdgtslkkqn tftlnlrevt
301 kdqsqkyccq vsndvgpgrs eevflqvqya pepstvqilh spavegsqve flcmslanpl
361 ptnytwyhng kemqgrteek vhipkilpwh agtyscvaen ilgtgqrgpg aeldvqyppk
421 kvttviqnpm piregdtvtl scnynssnps vtryewkphg aweepslgvl kiqnvgwdnt
481 tiacarcnsw cswaspvaln vqyaprdvrv rkikplseih sgnsvslqcd fssshpkevq
541 ffwekngrll gkesqlnfds ispedagsys cwvnnsigqt askawtlevl yaprrlrvsm
601 spqdqvmegk satltcesda nppvshytwf dwnnqslphh sqklrlepvk vqhsgaywcq
661 gtnsvgkgrs plstltvyys petigrrvav glgsclaili laicglklqr rwkrtqsqqg
721 lqenssgqsf fvrnkkvrra plsegphslg cynpmmedgi syttlrfpem niprtgdaes
781 semqrpprtc ddtvtysalh krqvgdyenv ipdfpedegi hyseliqfgv gerpqaqenv
841 dyvilkh  (SEQ ID NO: 27)
```

FIG. 1B

```
  1 mhligpwlll lvleylafsd sskwvfehpe tlyawegacv wipctyrald gdlesfilfh
 61 npeynkntsk fdgtrlyest kdgkvpseqk rvqflgdknk nctlsihpvh lndsgqlglr
121 mesktekwme rihlnvserp fpphiqlppe iqesqevtlt cllnfscygy piqlqwlleq
181 vpmrqaavts tsltiksvft rselkfspqw shhgkivtcq lqdadgkfls ndtvqlnvkh
241 ppkkvttviq npmpiregdt vtlscnynss npsvtryewk phgaweepsl gvlkiqnvgw
301 dnttiacaac nswcswaspv alnvqyaprd vrvrkikpls eihsgnsvsl qcdfssshpk
361 evqffwekng rllgkesqln fdsispedag syscwvnnsi gqtaskawtl evlyaprrlr
421 vsmspgdqvm egksatltce sdanppvshy twfdwnnqsl pyhsqklrle pvkvqhsgay
481 wcqgtnsvgk grsplstltv yyspetigrr vavglgscla ililaicglk lqrrwkrtqs
541 qqglqenssg qsffvrnkkv rraplsegph slgcynpmme dgisyttlrf pemniprtgd
601 aessemqrpp pdcddtvtys alhkrqvgtm rtsfqifqkm rgfitqs  (SEQ ID NO:29)
```

FIG. 1C

```
MHLLGPWLLLLEYLAFSDS SKWNIEHPGTIYAWEGACIWVPCTYRVLDGA
LETFILFHNPEYNQNMSKFEGTRLYESTKDGKVPSGQKRVQFLGNKINNNC
TLSIHPVHVNDSGQLGLRMVSKTEKWMERIHLNVSERPFPPRIQLPPKLQE
SQEVTLTCLLNFSCYGYQIQLQWLLEGAPMRQAAVTLTSLSTKSVFTRSEL
KFSPQWSHHGKIVTCELHDVDGKVLSEDTVQLNVKHTPKLTIEVTPNETIV
RKGDSVTMTCKVNSSNPEYTTVSWLKDDIPLKEQNTLMLTLHEVTKSQTGT
YCCRVSNDVGPATSEKVFLQVQYAPEPSRVQISQSPAVEGSEVNFLCISPA
NPLPTNYTWYHNGKEVQGRTEKQFQIQKILPWHAGTYSCVAENILGIGERG
PGTELDVQYPPKKVTMVIENPTPIREGDTVTLSCNYSSSNPIVNHYEWRPR
GAWEEPSLGVLKIQNIGWNNTAVACAACNNWCSWASPVTLNVLYAPRGVRV
RKIKPLSEIHSGNLVSLQCDFSSSHPKEVQFFWEKNGSLLGKESQLNFDSI
SPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLRVSMSQGNQVMEGKTA
ILTCESDANPPVYSYAWFDWNNQSLPYSGRMLRLEPVKVQHSGAYWCQGTN
RVGKGHSPLITLTVYYSPETIGRRVAVGLGSCLAILILAMCGFKVQRRWKR
TQSQQGLQENSSGQSFFVRNKKVRRTPLSEGPHSLGCYNPMMEDGISYATL
RFPETNTPRTGDAETSELQRLPPDCDDTVTYSVLQKRQVGDYENVIPDFPE
DEGIHYSELIQFGFGERPQAQENVDYVIVKH  (SEQ ID NO:31)
```

FIG. 1D

Heavy Chain Variable Domain
Sequence Alignment of Anti-CD22 10F4 Variants

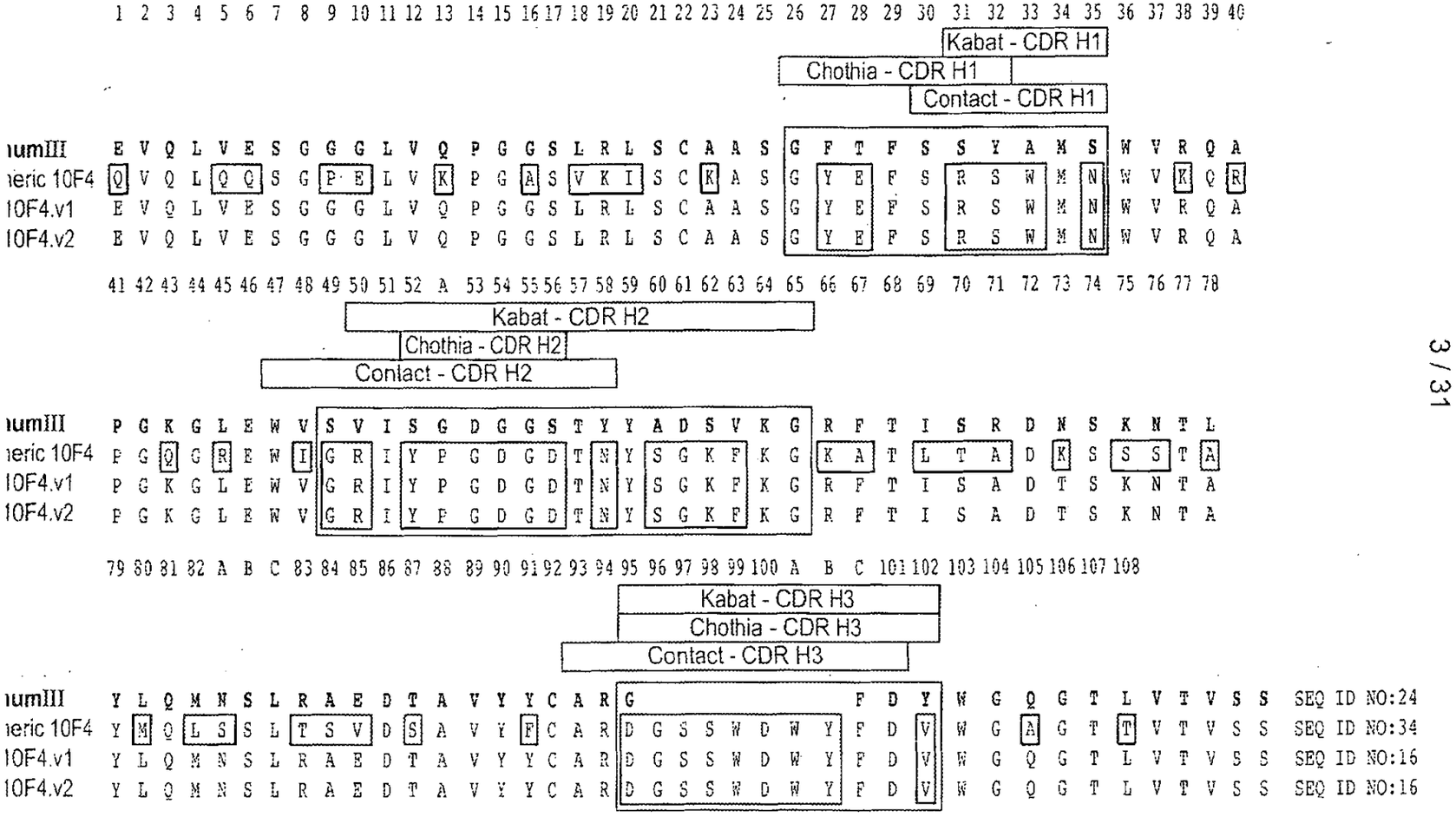


FIG. 2A

292

Light Chain Variable Domain
Sequence Alignment of Anti-CD22 10F4 Variants

Kabat - CDR L1; Chothia - CDR L1; Contact - CDR L1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	A	B	C	D	E	28	29	30	31	32	33	34	35	36	37
humKI	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	A	S	Q						S	I	S	N	Y	L	A	W	Y	Q
neric 10F4	D	I	L	M	T	Q	T	P	L	S	L	P	V	S	L	G	D	Q	A	S	I	S	C	R	S	S	Q	S	I	V	H	S	N	G	N	T	F	L	E	W	Y	L
10F4.v1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	S	S	Q	S	I	V	H	S	N	G	N	T	F	L	E	W	Y	Q
10F4.v2	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	I	T	C	R	S	S	Q	S	I	V	H	S	V	G	N	T	F	L	E	W	Y	Q

Kabat - CDR L2; Chothia - CDR L2; Contact - CDR L2

	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
humKI	Q	K	P	G	K	A	P	K	L	L	I	Y	A	A	S	S	L	E	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
neric 10F4	Q	K	P	G	Q	S	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V	E	A
10F4.v1	Q	K	P	G	K	A	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P
10F4.v2	Q	K	P	G	K	A	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P

Kabat - CDR L3; Chothia - CDR L3; Contact - CDR L3

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	
humKI	E	D	F	A	T	Y	Y	C	Q	Q	Y	N	S	L	P	W	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:25
neric 10F4	E	D	L	G	V	Y	Y	C	F	Q	G	S	Q	F	P	Y	T	F	G	G	G	T	K	V	E	I	K	SEQ ID NO:35
10F4.v1	E	D	F	A	T	Y	Y	C	F	Q	G	S	Q	F	P	Y	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:17
10F4.v2	E	D	F	A	T	Y	Y	C	F	Q	G	S	Q	F	P	Y	T	F	G	Q	G	T	K	V	E	I	K	SEQ ID NO:18

FIG. 2B

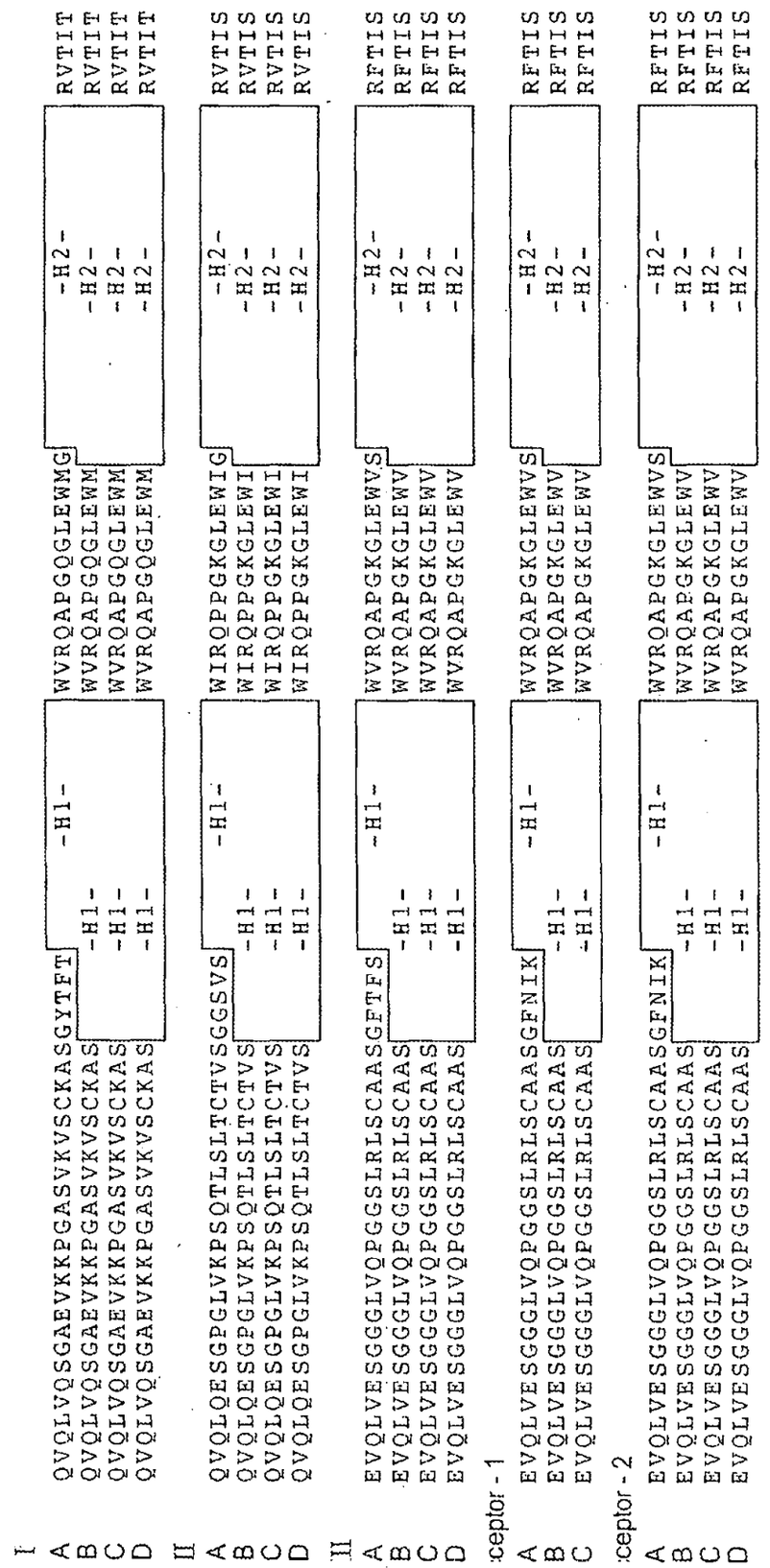

I
A QVQLVQSGAEVKKPGASVKVSCKASGYTFT -H1- WVRQAPGQGLEWMG -H2- RVTIT
B QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
C QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
D QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
II
A QVQLQESGPGLVKPSQTLSLTCTVSGGSVS -H1- WIRQPPGKGLEWIG -H2- RVTIS
B QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
C QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
D QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
III
A EVQLVESGGGLVQPGGSLRLSCAASGFTFS -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Receptor - 1
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Receptor - 2
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS

FIG. 3A

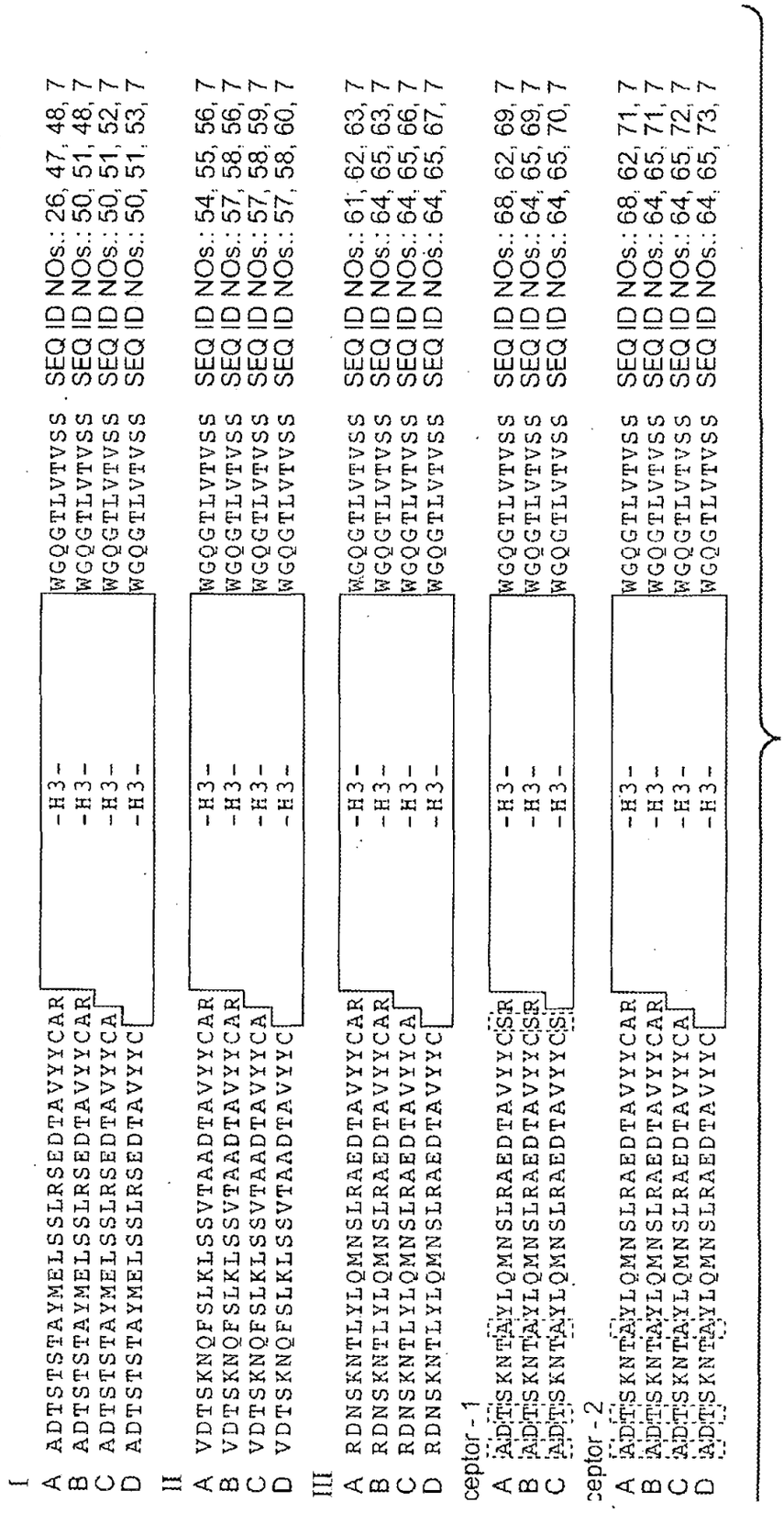

I
A ADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 26, 47, 48, 7
B ADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 50, 51, 48, 7
C ADTSTSTAYMELSSLRSEDTAVYYCA -H3- WGQGTLVTVSS SEQ ID NOs.: 50, 51, 52, 7
D ADTSTSTAYMELSSLRSEDTAVYYC -H3- WGQGTLVTVSS SEQ ID NOs.: 50, 51, 53, 7
II
A VDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 54, 55, 56, 7
B VDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 57, 58, 56, 7
C VDTSKNQFSLKLSSVTAADTAVYYCA -H3- WGQGTLVTVSS SEQ ID NOs.: 57, 58, 59, 7
D VDTSKNQFSLKLSSVTAADTAVYYC -H3- WGQGTLVTVSS SEQ ID NOs.: 57, 58, 60, 7
III
A RDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 61, 62, 63, 7
B RDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 63, 7
C RDNSKNTLYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 66, 7
D RDNSKNTLYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 67, 7
ceptor - 1
A ADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS SEQ ID NOs.: 68, 62, 69, 7
B ADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 69, 7
C ADTSKNTAYLQMNSLRAEDTAVYYCS -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 70, 7
ceptor - 2
A ADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 68, 62, 71, 7
B ADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 71, 7
C ADTSKNTAYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 72, 7
D ADTSKNTAYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS SEQ ID NOs.: 64, 65, 73, 7

FIG. 3B

kv1	DIQMTQSPSSLSASVGDRVTITC	-L1-	WYQQKPGKAPKLLIY	-L2- GVPSRFSGSGSGTDFTLTISSLQP
kv1	DIQMTQSPSSLSASVGDRVTITC	-L1-	WYQQKPGKAPKLLI	-L2- GVPSRFSGSGSGTDFTLTISSLQP
kv2	DIVMTQSPLSLPVTPGEPASISC	-L1-	WYLQKPGQSPQLLIY	-L2- GVPDRFSGSGSGTDFTLKISRVEA
kv3	EIVLTQSPGTLSLSPGERATLSC	-L1-	WYQQKPGQAPRLLIY	-L2- GIPDRFSGSGSGTDFTLTISRLEP
kv4	DIVMTQSPDSLAVSLGERATINC	-L1-	WYQQKPGQPPKLLIY	-L2- GVPDRFSGSGSGTDFTLTISSLQA

FIG. 4A

kv1	EDFATYYC	-L3-	FGQGTKVEIK SEQ ID NOs.: 74, 75, 76, 77
kv1	EDFATYYC	-L3-	FGQGTKVEIK SEQ ID NOs.: 74, 78, 76, 77
kv2	EDVGVYYC	-L3-	FGQGTKVEIK SEQ ID NOs.: 49, 79, 80, 77
kv3	EDFAVYYC	-L3-	FGQGTKVEIK SEQ ID NOs.: 81, 82, 83, 77
kv4	EDVAVYYC	-L3-	FGQGTKVEIK SEQ ID NOs.: 84, 85, 86, 77

FIG. 4B

```
          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.: 38
humIgG2     EALHNHYTQKSLSLSPGK  SEQ ID NO.: 39
humIgG3     EALHNRFTQKSLSLSPGK  SEQ ID NO.: 40
humIgG4     EALHNHYTQKSLSLSLGK  SEQ ID NO.: 41
                 **         *
```

A Kappa Light Chain Constant Region Consensus Sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC SEQ ID NO: 37

FIG. 5A

Anti-CD22 h10F4v2 Light Chain

DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKPGKAPKLLI
YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSQFPYTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC (SEQ ID NO:87)

Anti-CD22 h10F4v2 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:88)

FIG. 5B

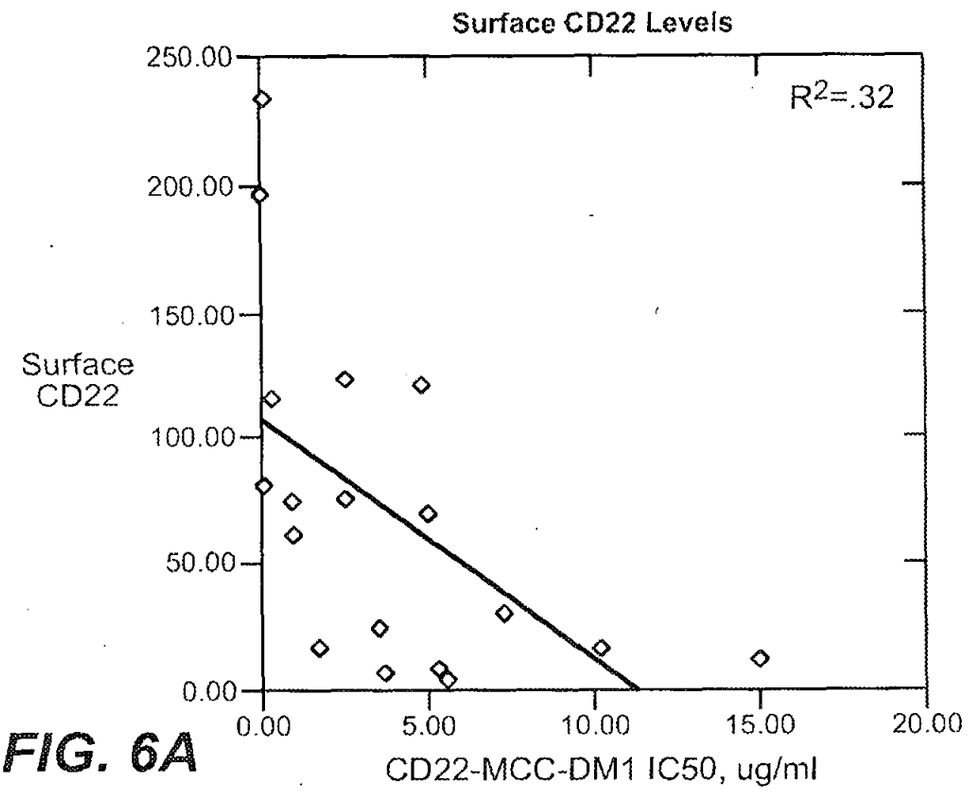

Surface CD22 Levels
R^2=.32
250.00
200.00
150.00
100.00
50.00
0.00
Surface CD22
0.00
5.00
10.00
15.00
20.00
CD22-MCC-DM1 IC50, ug/ml

FIG. 6A

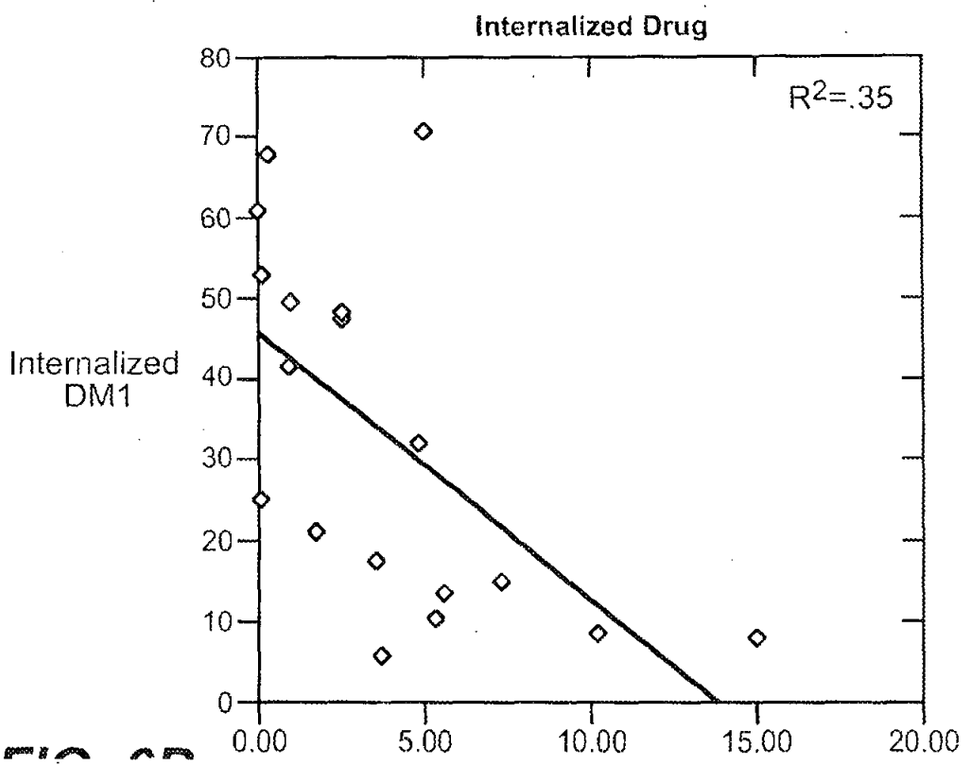

Internalized Drug
R^2=.35
80
70
60
50
40
30
20
10
0
Internalized DM1
0.00
5.00
10.00
15.00
20.00

FIG. 6B

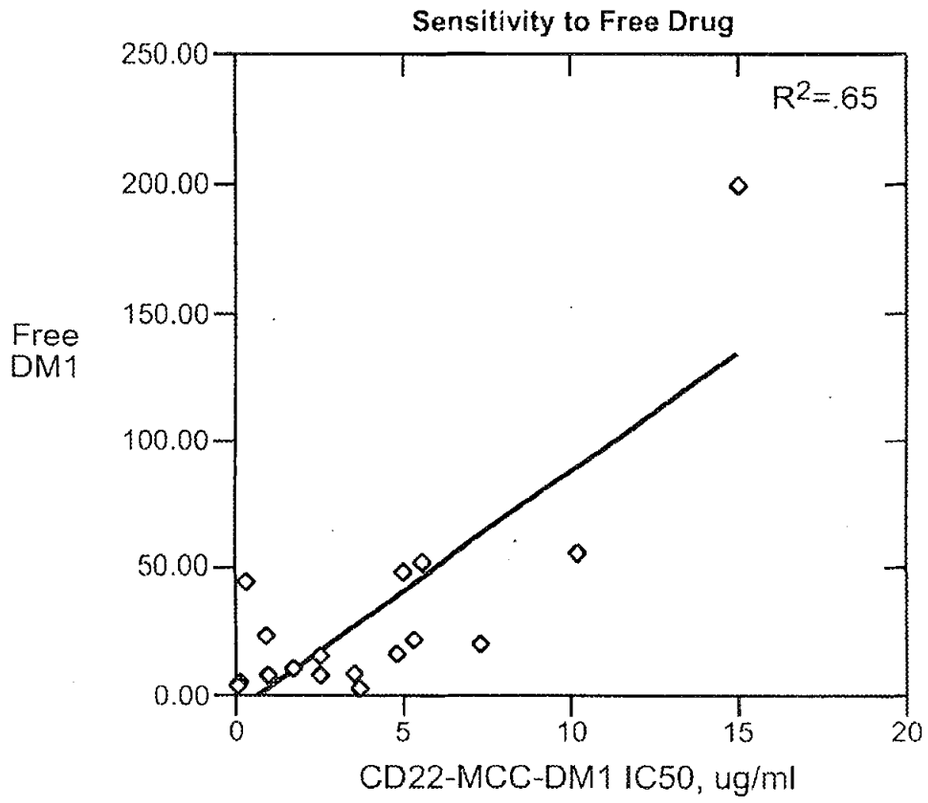
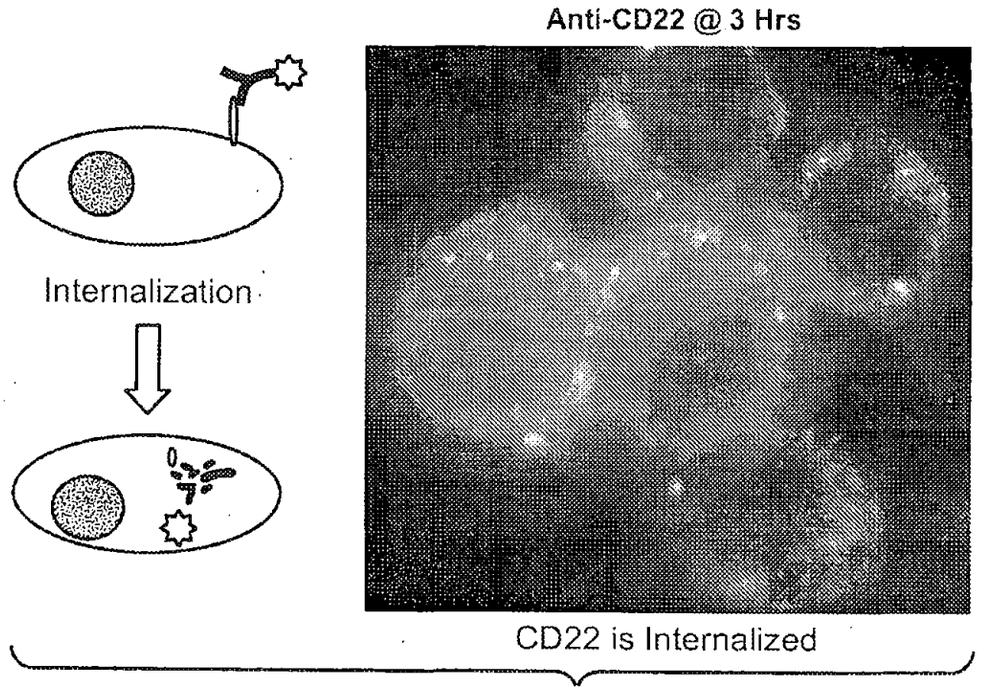

Sensitivity to Free Drug
250.00
200.00
150.00
100.00
50.00
0.00
Free DM1
R2=.65
0
5
10
15
20
CD22-MCC-DM1 IC50, ug/ml

FIG. 6C


Anti-CD22 @ 3 Hrs
Internalization
CD22 is Internalized

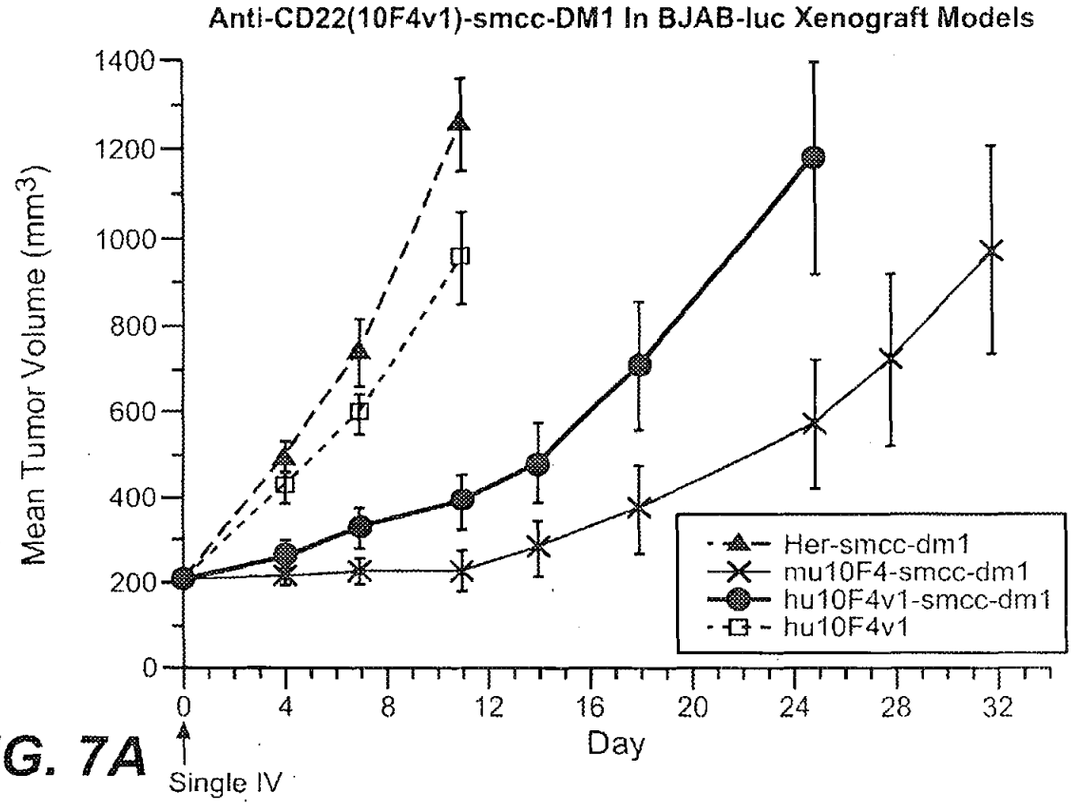
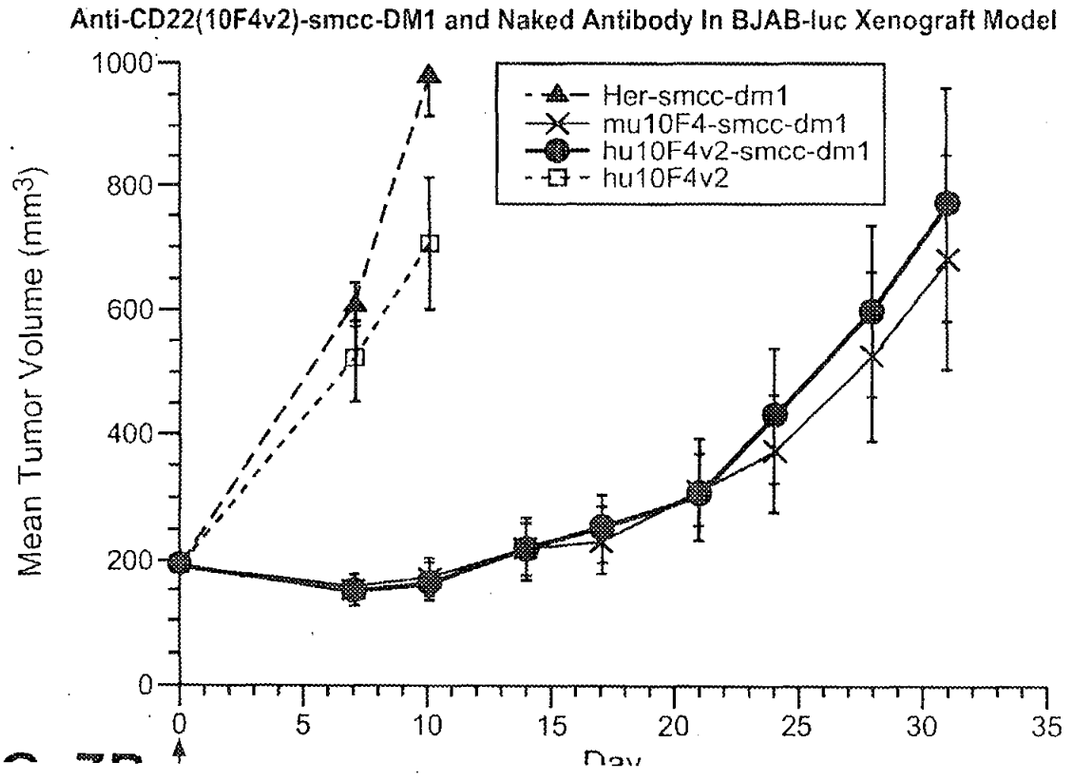

Anti-CD22(10F4v1)-smcc-DM1 In BJAB-luc Xenograft Models
Mean Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
0
4
8
12
16
20
24
28
32
Day
Single IV
Her-smcc-dm1
mu10F4-smcc-dm1
hu10F4v1-smcc-dm1
hu10F4v1

FIG. 7A


Anti-CD22(10F4v2)-smcc-DM1 and Naked Antibody In BJAB-luc Xenograft Model
Mean Tumor Volume (mm3)
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
Day
Her-smcc-dm1
mu10F4-smcc-dm1
hu10F4v2-smcc-dm1
hu10F4v2

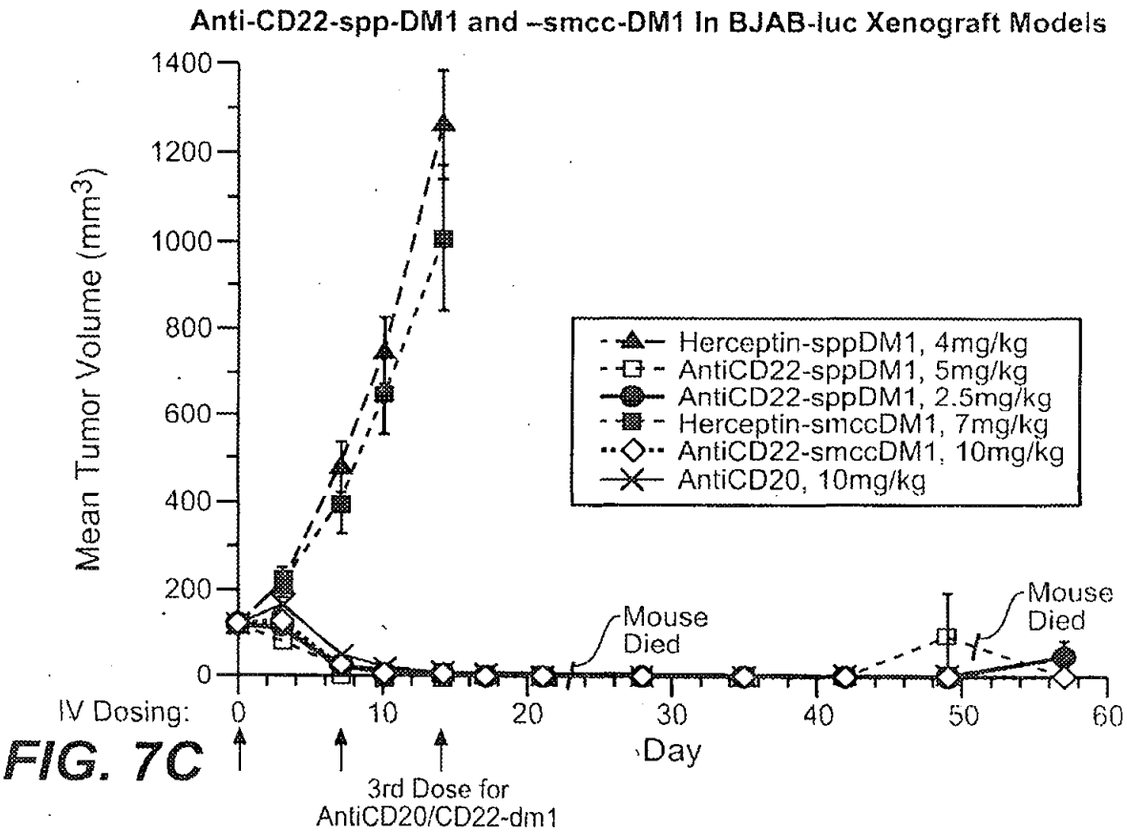

Anti-CD22-spp-DM1 and –smcc-DM1 In BJAB-luc Xenograft Models
Mean Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
Herceptin-sppDM1, 4mg/kg
AntiCD22-sppDM1, 5mg/kg
AntiCD22-sppDM1, 2.5mg/kg
Herceptin-smccDM1, 7mg/kg
AntiCD22-smccDM1, 10mg/kg
AntiCD20, 10mg/kg
Mouse Died
Mouse Died
IV Dosing:
0
10
20
30
40
50
60
Day
3rd Dose for AntiCD20/CD22-dm1

FIG. 7C

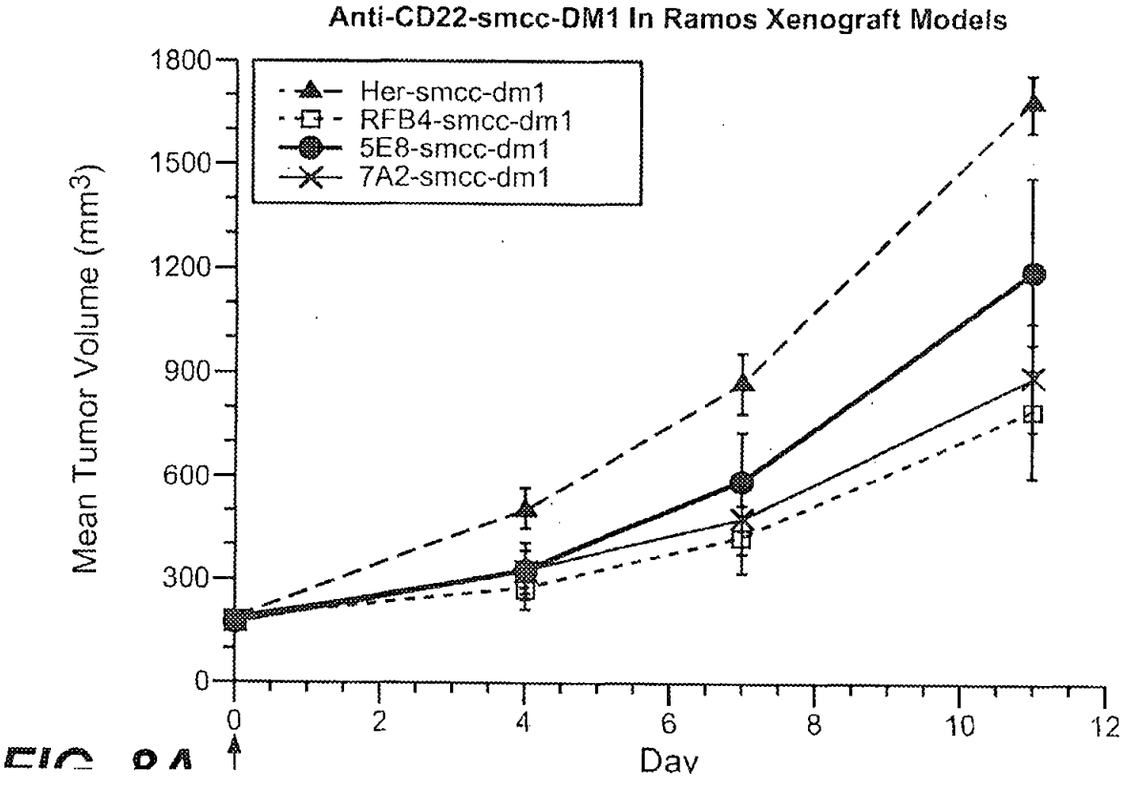

Anti-CD22-smcc-DM1 In Ramos Xenograft Models
Mean Tumor Volume (mm3)
1800
1500
1200
900
600
300
0
Her-smcc-dm1
RFB4-smcc-dm1
5E8-smcc-dm1
7A2-smcc-dm1
0
2
4
6
8
10
12
Day

FIG. 8A

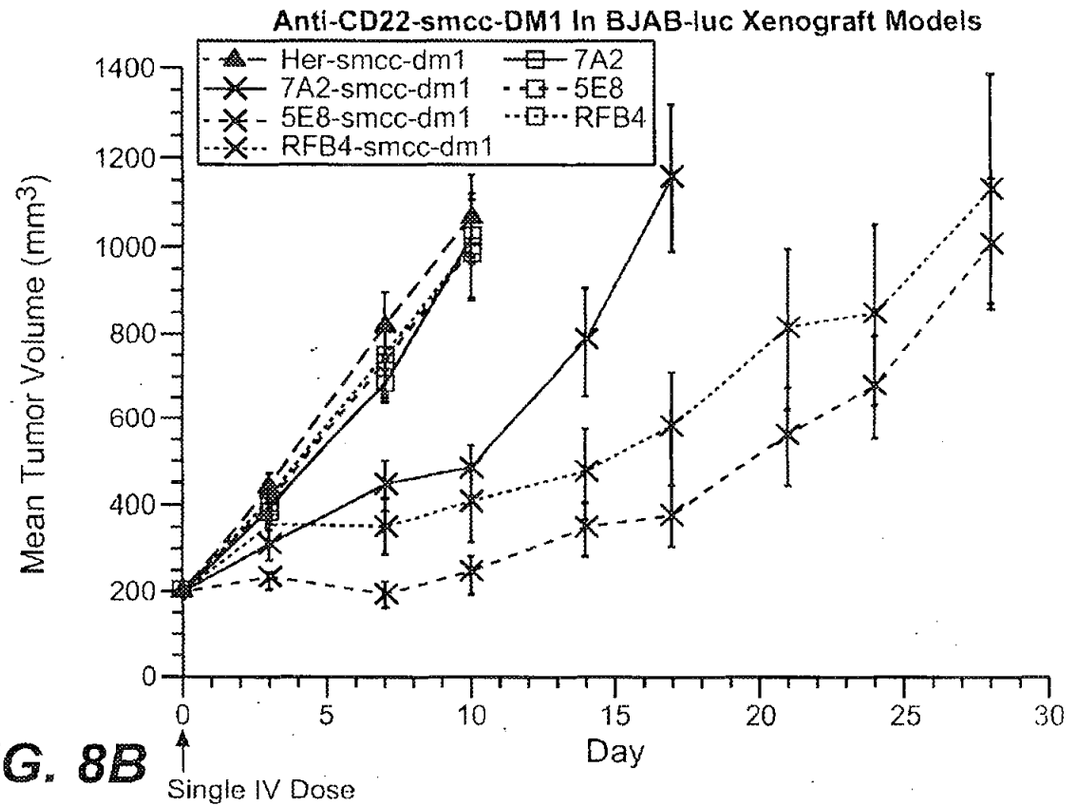

Anti-CD22-smcc-DM1 In BJAB-luc Xenograft Models
Her-smcc-dm1
7A2-smcc-dm1
5E8-smcc-dm1
RFB4-smcc-dm1
7A2
5E8
RFB4
Mean Tumor Volume (mm3)
Day
Single IV Dose

FIG. 8B

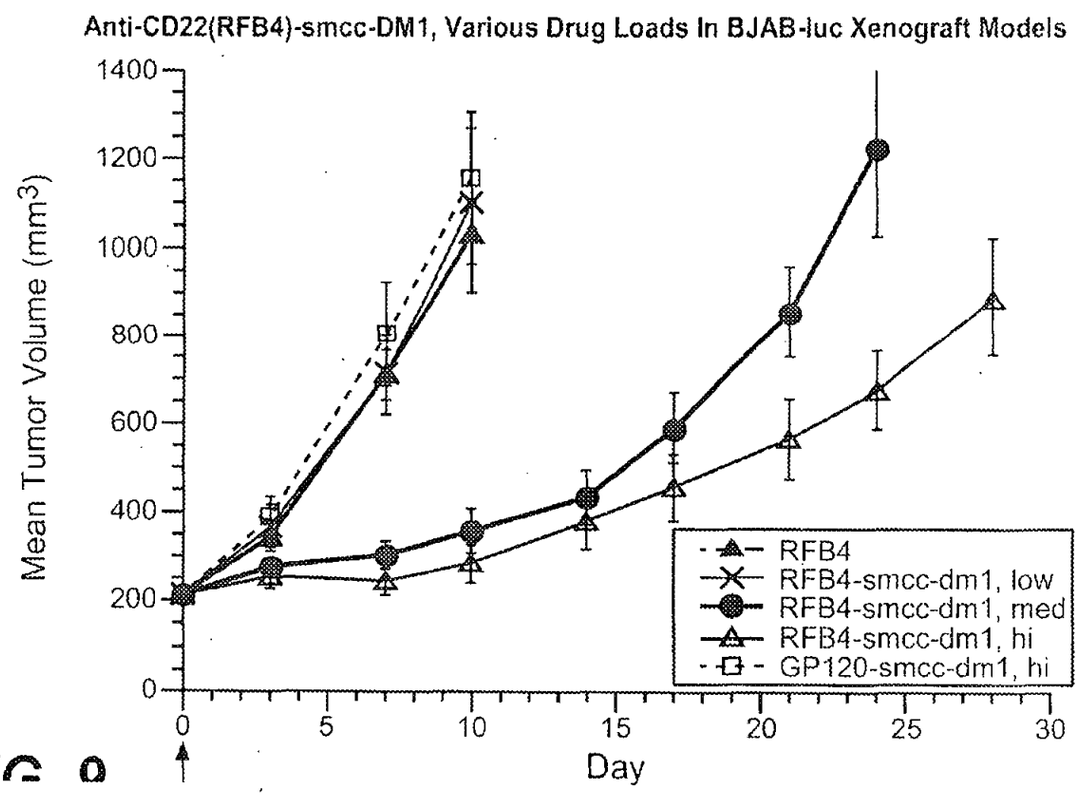

Anti-CD22(RFB4)-smcc-DM1, Various Drug Loads In BJAB-luc Xenograft Models
RFB4
RFB4-smcc-dm1, low
RFB4-smcc-dm1, med
RFB4-smcc-dm1, hi
GP120-smcc-dm1, hi
Mean Tumor Volume (mm3)
Day

FIG. 9

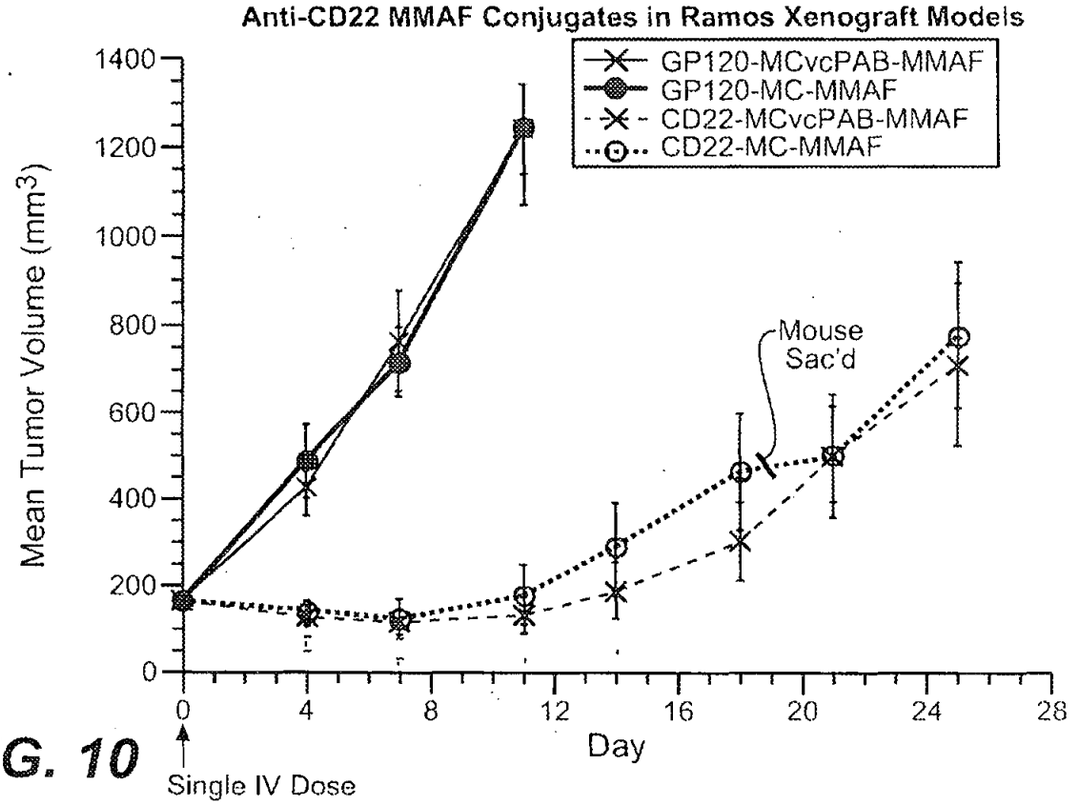

Anti-CD22 MMAF Conjugates in Ramos Xenograft Models
GP120-MCvcPAB-MMAF
GP120-MC-MMAF
CD22-MCvcPAB-MMAF
CD22-MC-MMAF
Mouse Sac'd
Mean Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
0
4
8
12
16
20
24
28
Day
Single IV Dose

FIG. 10

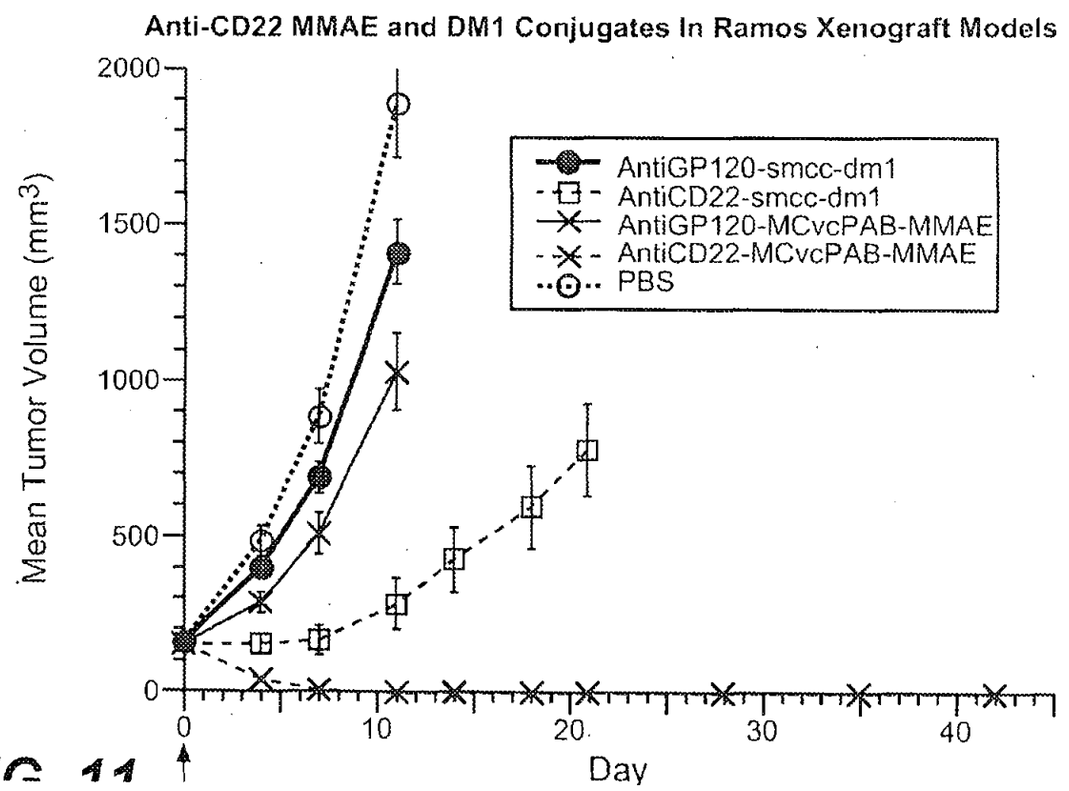

Anti-CD22 MMAE and DM1 Conjugates In Ramos Xenograft Models
AntiGP120-smcc-dm1
AntiCD22-smcc-dm1
AntiGP120-MCvcPAB-MMAE
AntiCD22-MCvcPAB-MMAE
PBS
Mean Tumor Volume (mm3)
2000
1500
1000
500
0
0
10
20
30
40
Day

FIG. 11

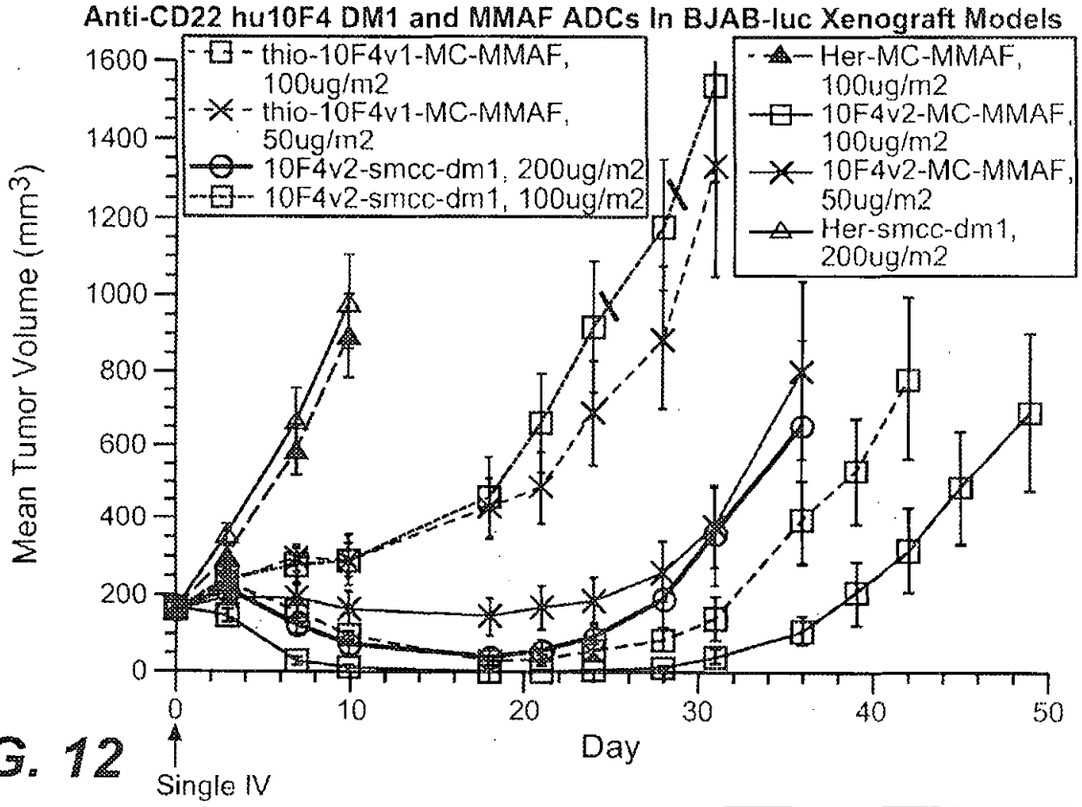


FIG. 12

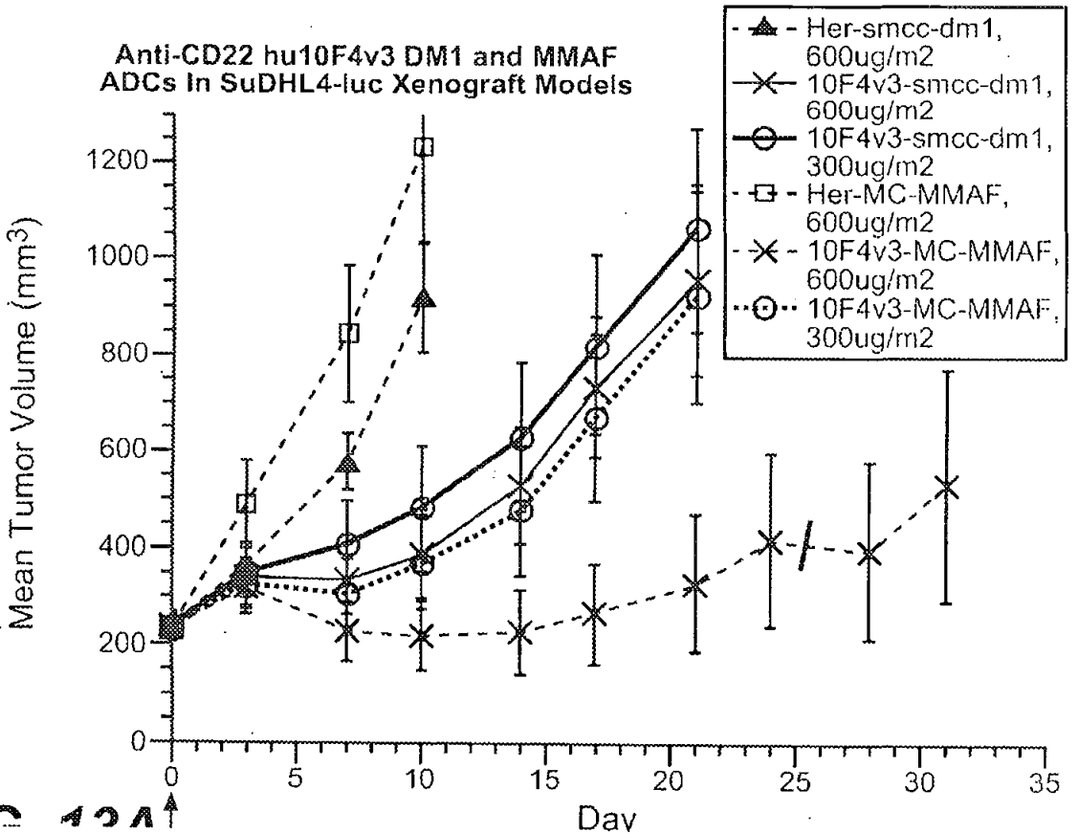


FIG. 13A

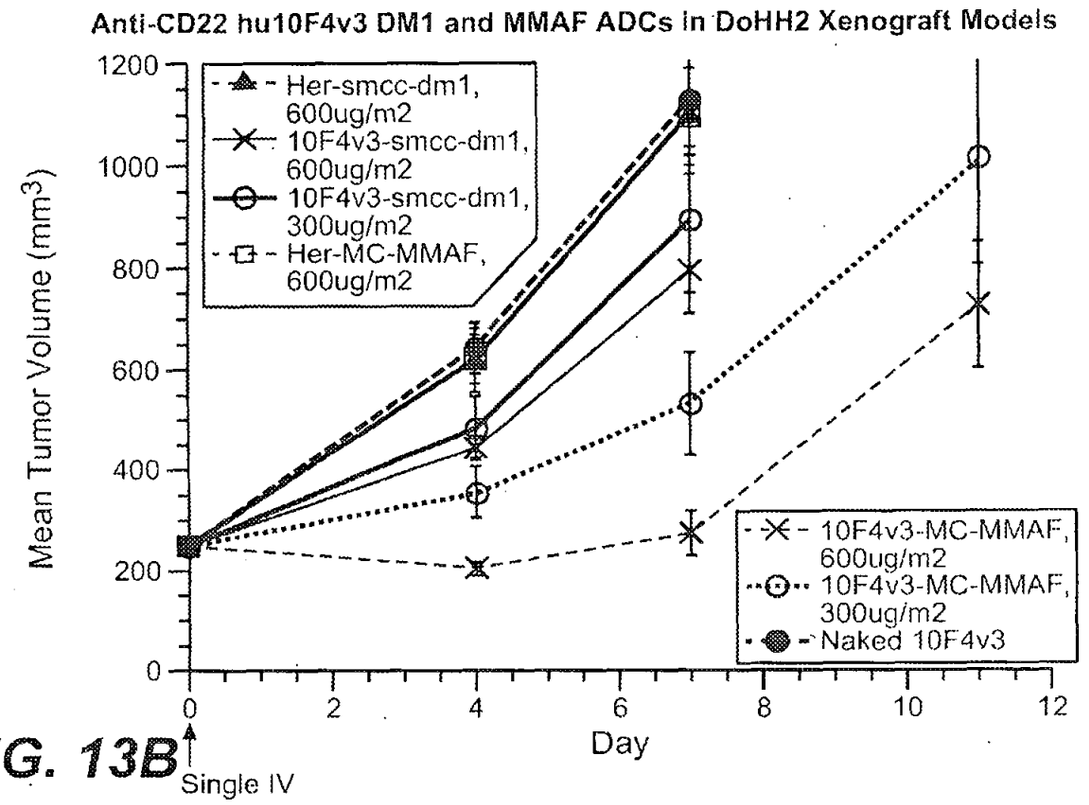


FIG. 13B

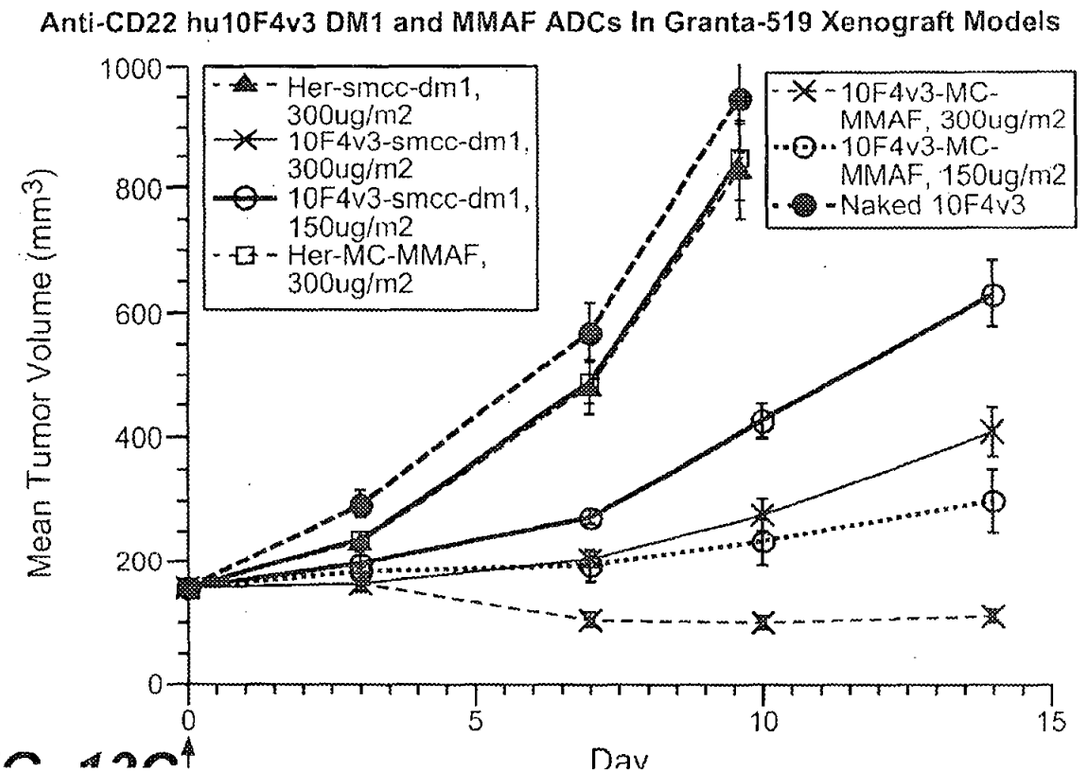


FIG. 13C

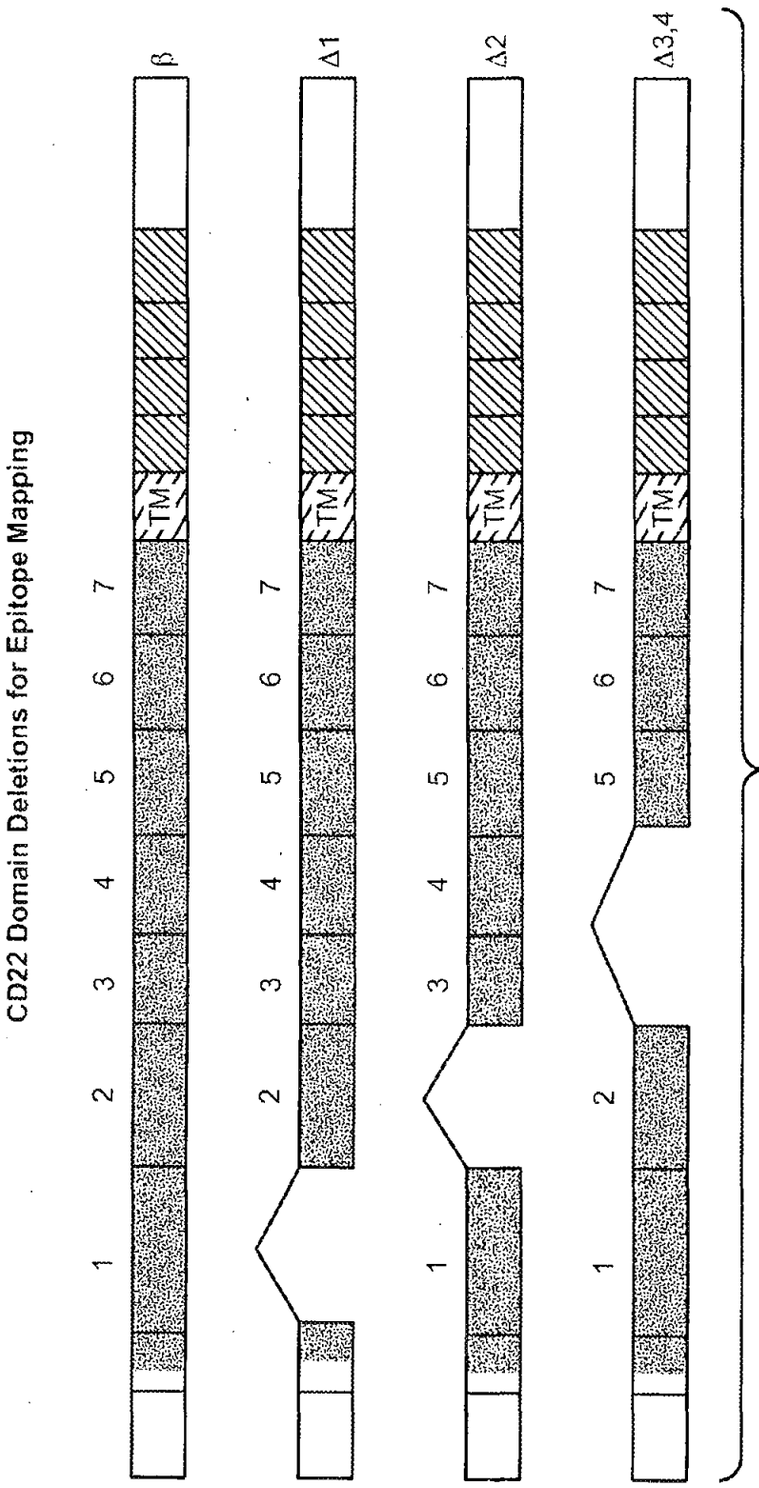

CD22 Domain Deletions for Epitope Mapping
β
Δ1
Δ2
Δ3,4
TM
1
2
3
4
5
6
7

FIG. 14

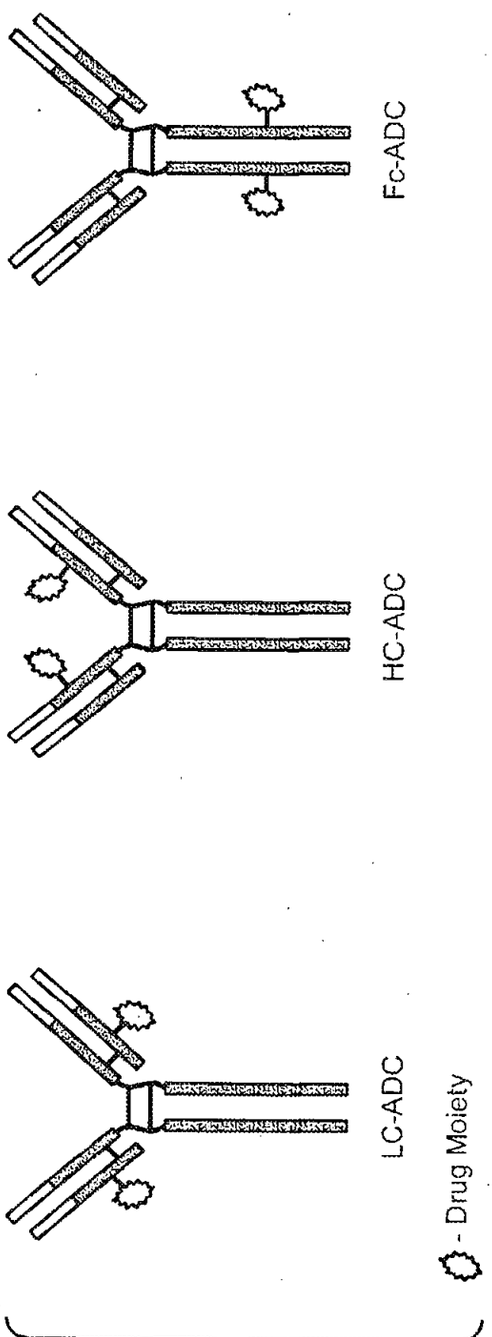

LC-ADC
HC-ADC
Fc-ADC
- Drug Moiety

FIG. 15

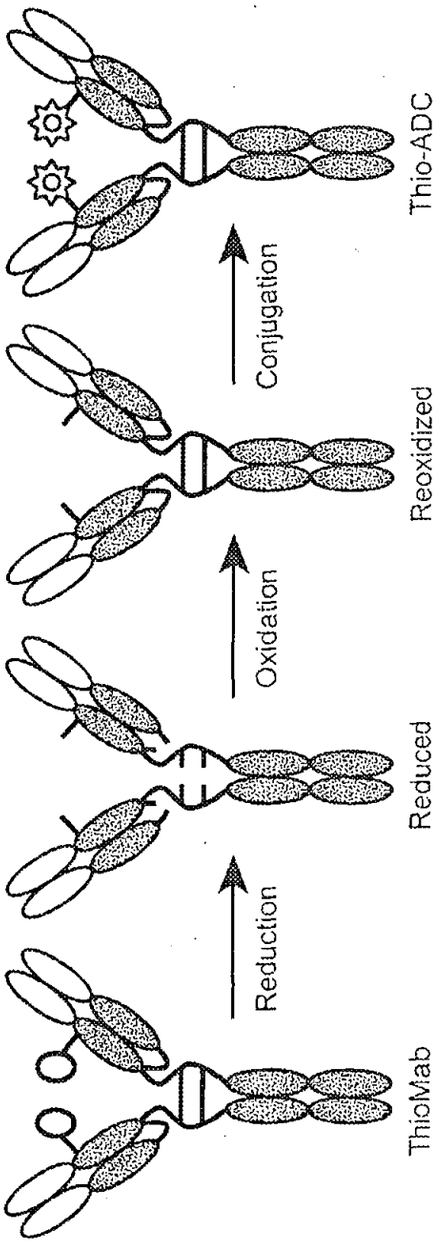

ThioMab
Reduction
Reduced
Oxidation
Reoxidized
Conjugation
Thio-ADC

FIG. 16

Anti-CD22 V205C h10F4v3 Cysteine Engineered Light Chain

DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKPGKAPKLLI
YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSQFPYTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTK
SFNRGEC (SEQ ID NO:91)

FIG. 17A

Anti-CD22 A118C h10F4v3 Cysteine Engineered Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:92)

FIG. 17B

Anti-CD22 S400C h10F4v3 Cysteine Engineered Fc Region

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:93)

FIG. 17C

FIG. 18A

FIG. 18B

FIG. 18C

FIG. 18D

FIG. 18E

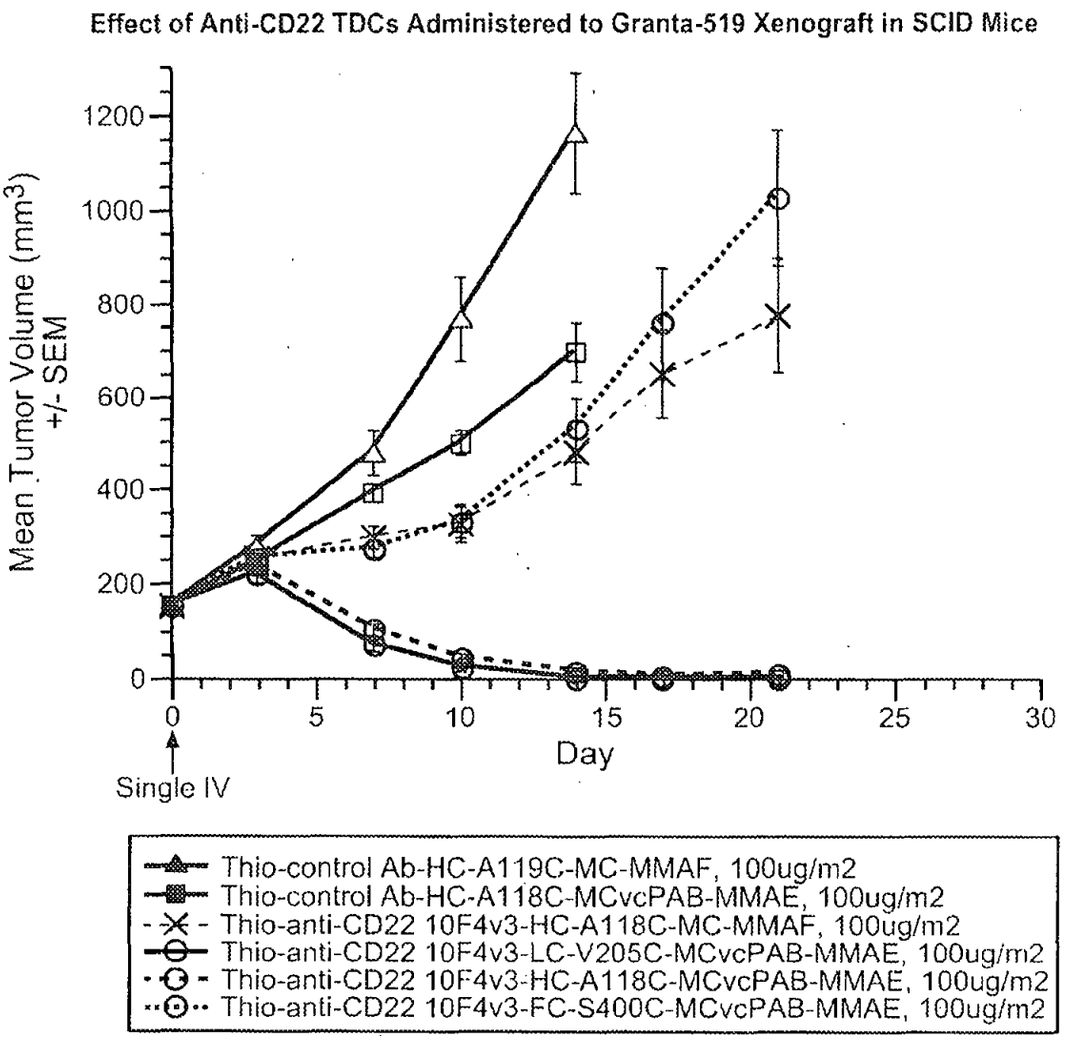

Effect of Anti-CD22 TDCs Administered to Granta-519 Xenograft in SCID Mice
Mean Tumor Volume (mm3)
+/- SEM
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Day
Single IV
Thio-control Ab-HC-A119C-MC-MMAF, 100ug/m2
Thio-control Ab-HC-A118C-MCvcPAB-MMAE, 100ug/m2
Thio-anti-CD22 10F4v3-HC-A118C-MC-MMAF, 100ug/m2
Thio-anti-CD22 10F4v3-LC-V205C-MCvcPAB-MMAE, 100ug/m2
Thio-anti-CD22 10F4v3-HC-A118C-MCvcPAB-MMAE, 100ug/m2
Thio-anti-CD22 10F4v3-FC-S400C-MCvcPAB-MMAE, 100ug/m2

FIG. 19

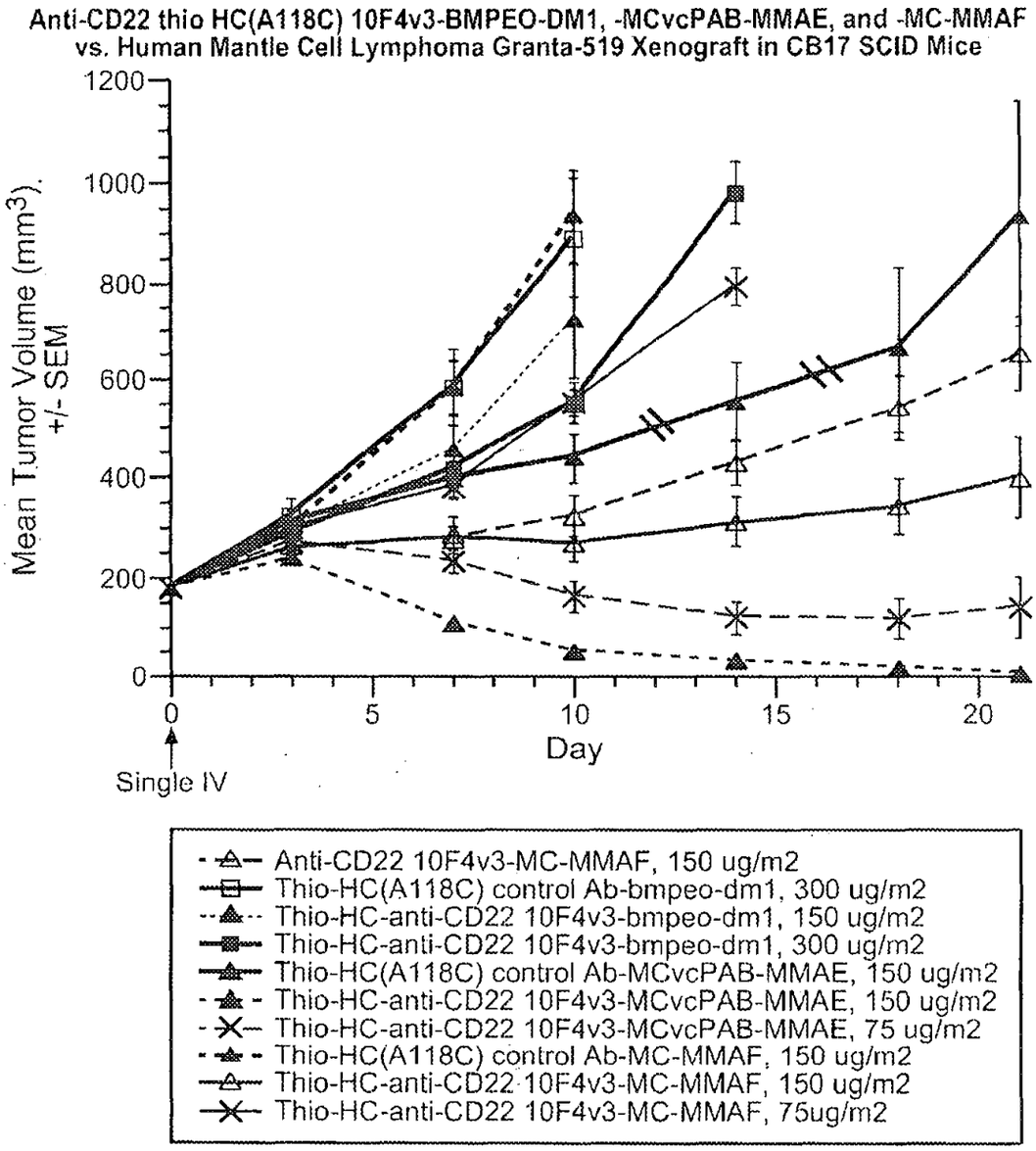

Anti-CD22 thio HC(A118C) 10F4v3-BMPEO-DM1, -MCvcPAB-MMAE, and -MC-MMAF
vs. Human Mantle Cell Lymphoma Granta-519 Xenograft in CB17 SCID Mice
Mean Tumor Volume (mm3), +/- SEM
1200
1000
800
600
400
200
0
0
5
10
15
20
Day
Single IV
Anti-CD22 10F4v3-MC-MMAF, 150 ug/m2
Thio-HC(A118C) control Ab-bmpeo-dm1, 300 ug/m2
Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 150 ug/m2
Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 300 ug/m2
Thio-HC(A118C) control Ab-MCvcPAB-MMAE, 150 ug/m2
Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 150 ug/m2
Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 75 ug/m2
Thio-HC(A118C) control Ab-MC-MMAF, 150 ug/m2
Thio-HC-anti-CD22 10F4v3-MC-MMAF, 150 ug/m2
Thio-HC-anti-CD22 10F4v3-MC-MMAF, 75ug/m2

FIG. 20A

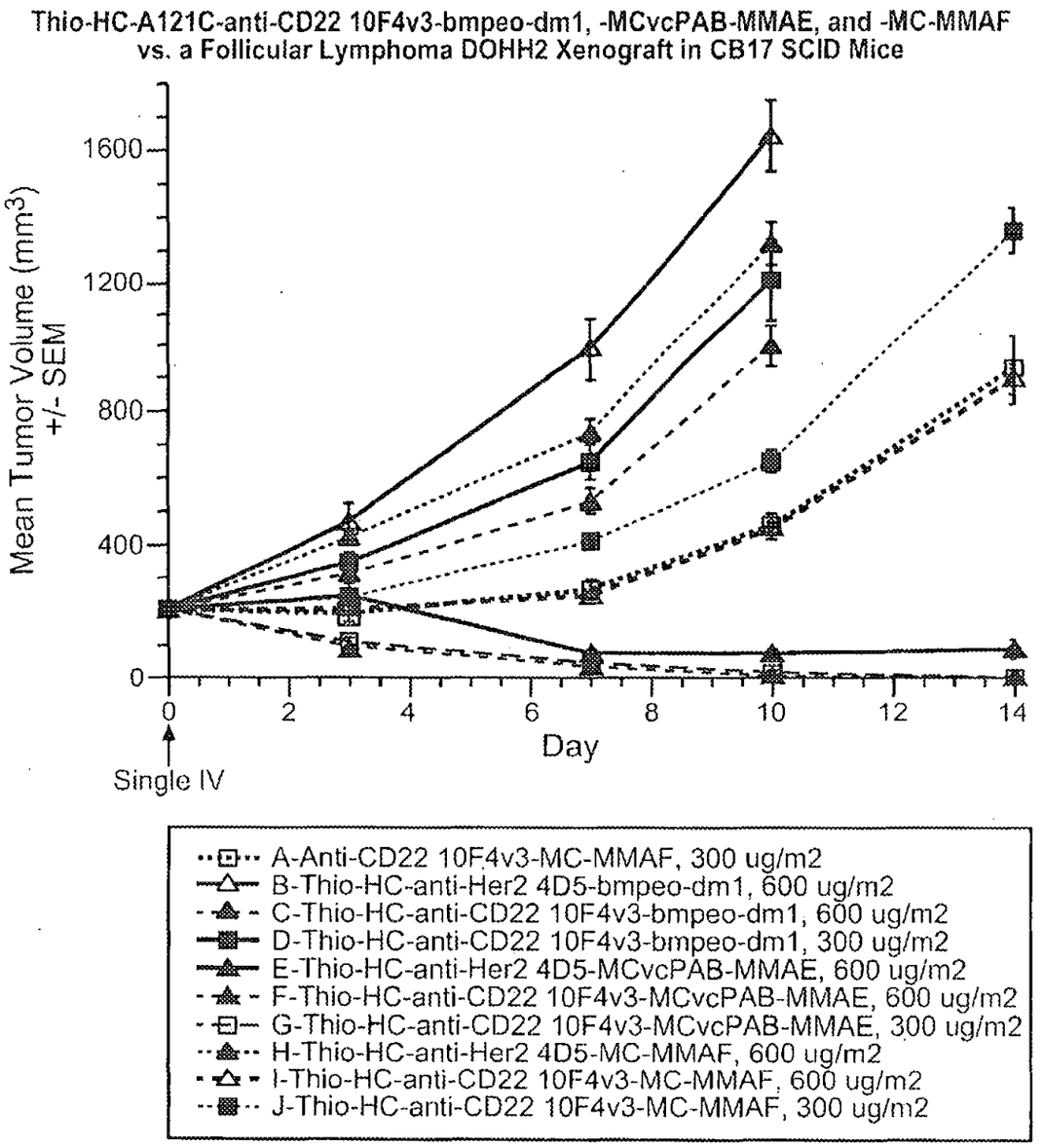

Thio-HC-A121C-anti-CD22 10F4v3-bmpeo-dm1, -MCvcPAB-MMAE, and -MC-MMAF
vs. a Follicular Lymphoma DOHH2 Xenograft in CB17 SCID Mice
Mean Tumor Volume (mm3)
+/- SEM
1600
1200
800
400
0
0
2
4
6
8
10
12
14
Day
Single IV
A-Anti-CD22 10F4v3-MC-MMAF, 300 ug/m2
B-Thio-HC-anti-Her2 4D5-bmpeo-dm1, 600 ug/m2
C-Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 600 ug/m2
D-Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 300 ug/m2
E-Thio-HC-anti-Her2 4D5-MCvcPAB-MMAE, 600 ug/m2
F-Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 600 ug/m2
G-Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 300 ug/m2
H-Thio-HC-anti-Her2 4D5-MC-MMAF, 600 ug/m2
I-Thio-HC-anti-CD22 10F4v3-MC-MMAF, 600 ug/m2
J-Thio-HC-anti-CD22 10F4v3-MC-MMAF, 300 ug/m2

FIG. 20B

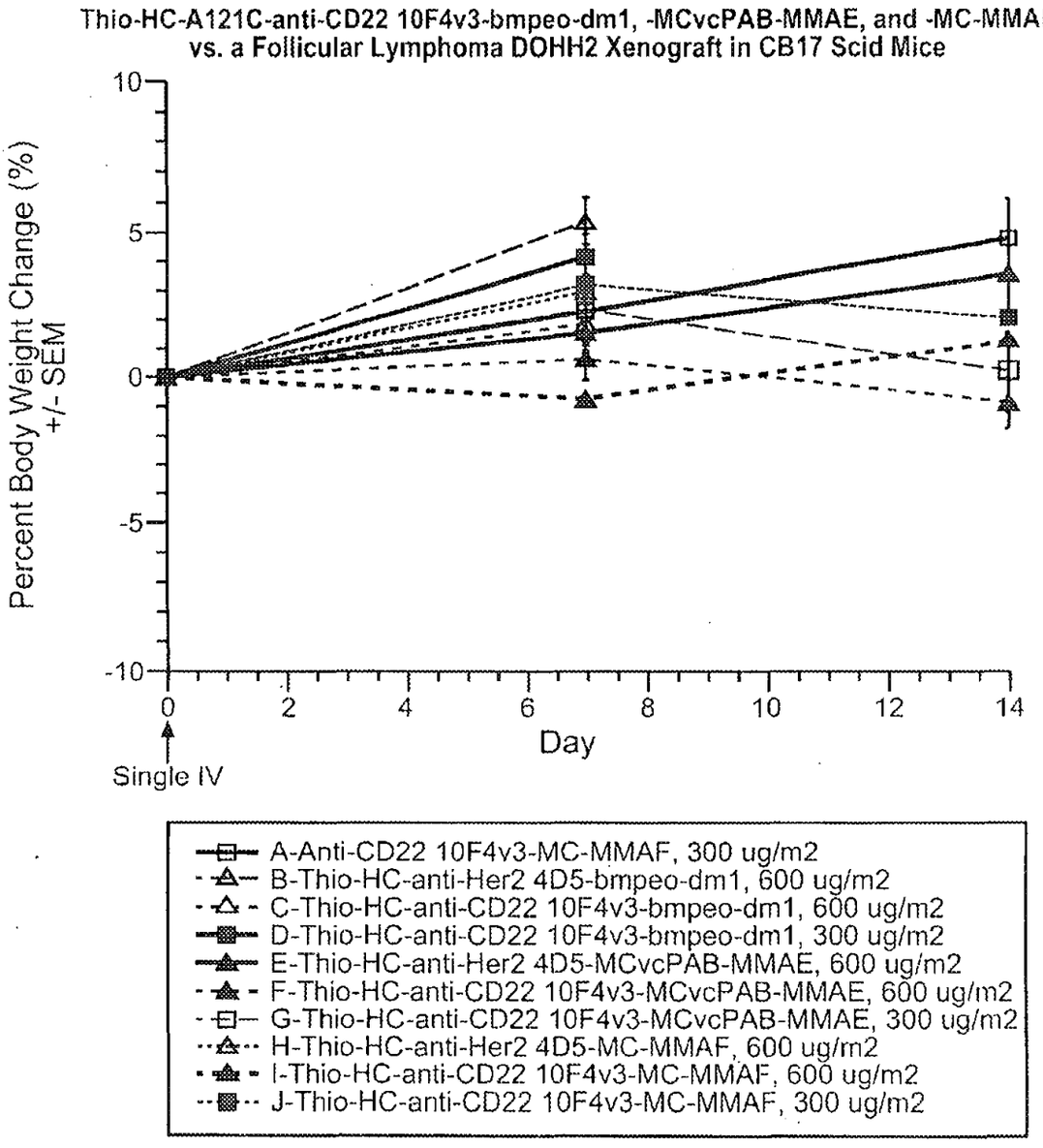

Thio-HC-A121C-anti-CD22 10F4v3-bmpeo-dm1, -MCvcPAB-MMAE, and -MC-MMAF
vs. a Follicular Lymphoma DOHH2 Xenograft in CB17 Scid Mice
Percent Body Weight Change (%)
+/- SEM
10
5
0
-5
-10
0
2
4
6
8
10
12
14
Day
Single IV
A-Anti-CD22 10F4v3-MC-MMAF, 300 ug/m2
B-Thio-HC-anti-Her2 4D5-bmpeo-dm1, 600 ug/m2
C-Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 600 ug/m2
D-Thio-HC-anti-CD22 10F4v3-bmpeo-dm1, 300 ug/m2
E-Thio-HC-anti-Her2 4D5-MCvcPAB-MMAE, 600 ug/m2
F-Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 600 ug/m2
G-Thio-HC-anti-CD22 10F4v3-MCvcPAB-MMAE, 300 ug/m2
H-Thio-HC-anti-Her2 4D5-MC-MMAF, 600 ug/m2
I-Thio-HC-anti-CD22 10F4v3-MC-MMAF, 600 ug/m2
J-Thio-HC-anti-CD22 10F4v3-MC-MMAF, 300 ug/m2

FIG. 20C

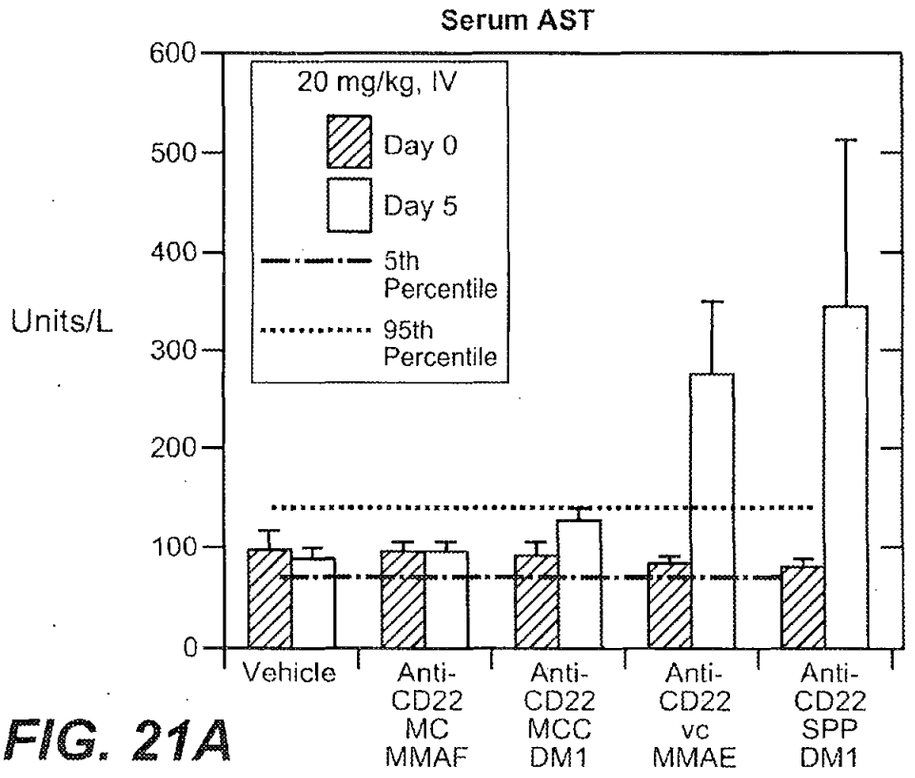
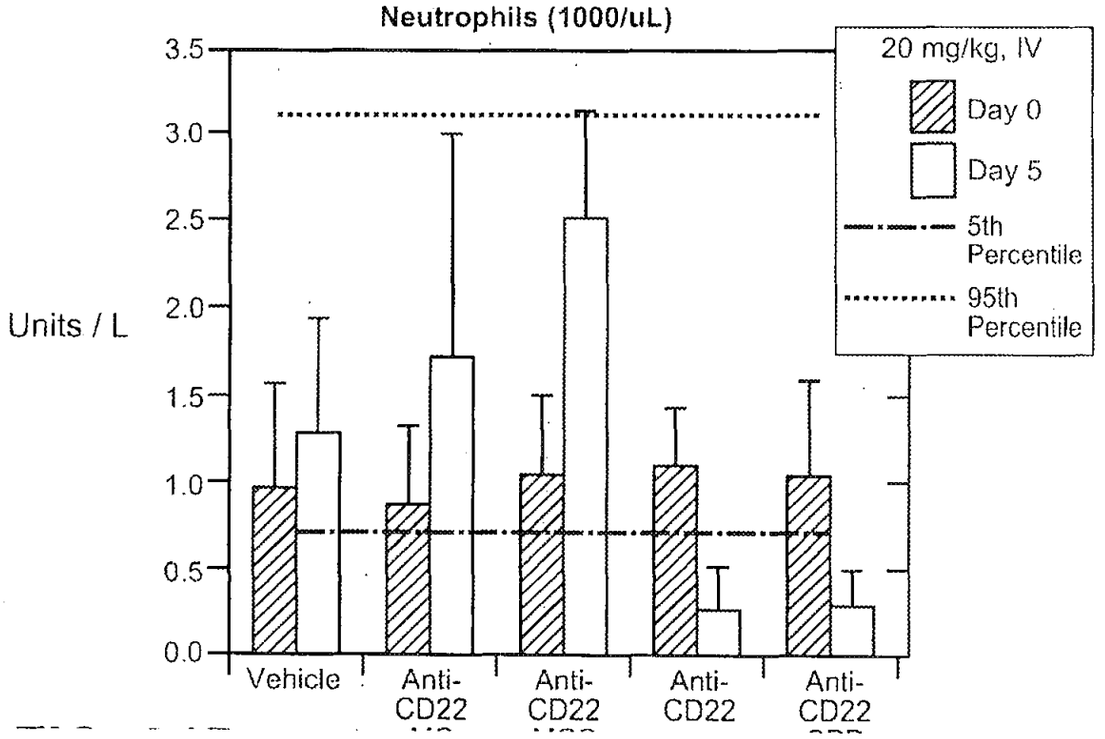

Serum AST
20 mg/kg, IV
Day 0
Day 5
5th Percentile
95th Percentile
Units/L
600
500
400
300
200
100
0
Vehicle
Anti-CD22 MC MMAF
Anti-CD22 MCC DM1
Anti-CD22 vc MMAE
Anti-CD22 SPP DM1

FIG. 21A


Neutrophils (1000/uL)
20 mg/kg, IV
Day 0
Day 5
5th Percentile
95th Percentile
Units / L
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Vehicle
Anti-CD22
Anti-CD22
Anti-CD22
Anti-CD22

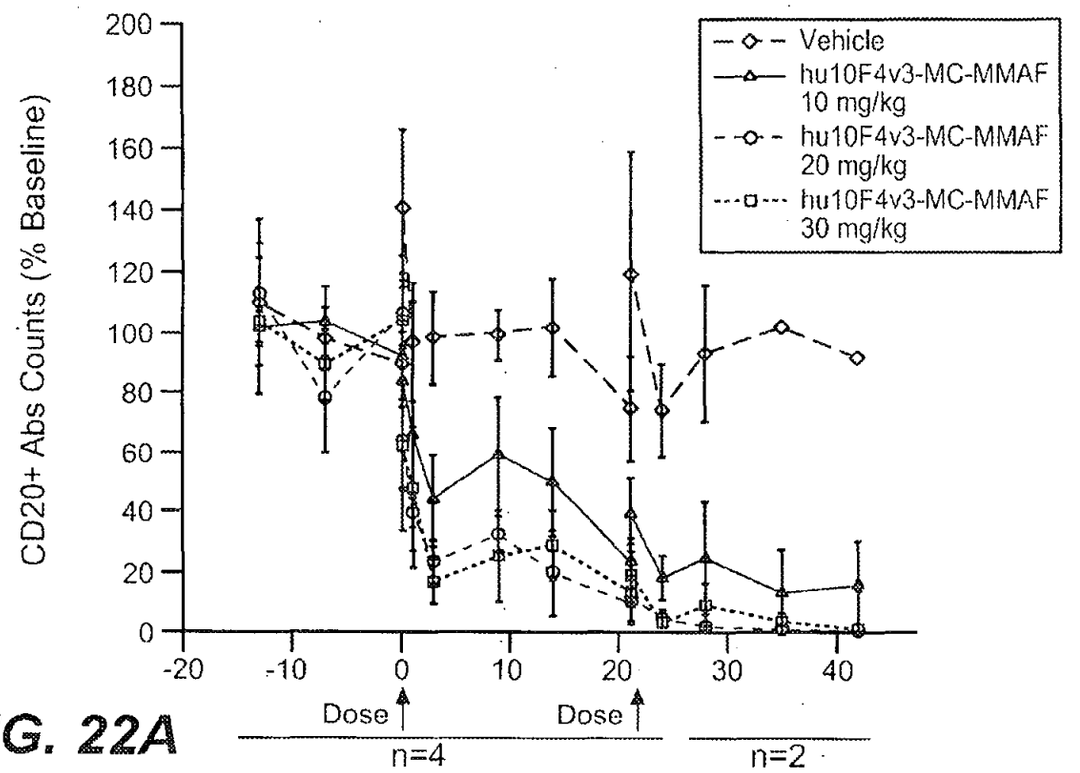


FIG. 22A

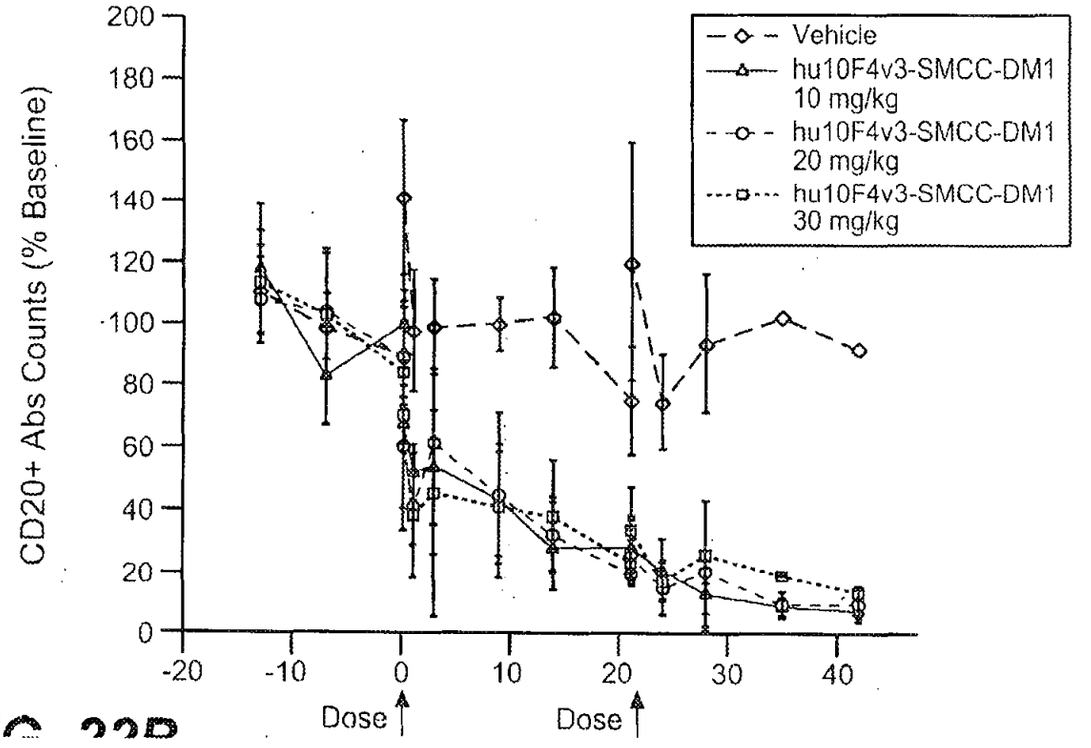


FIG. 22B

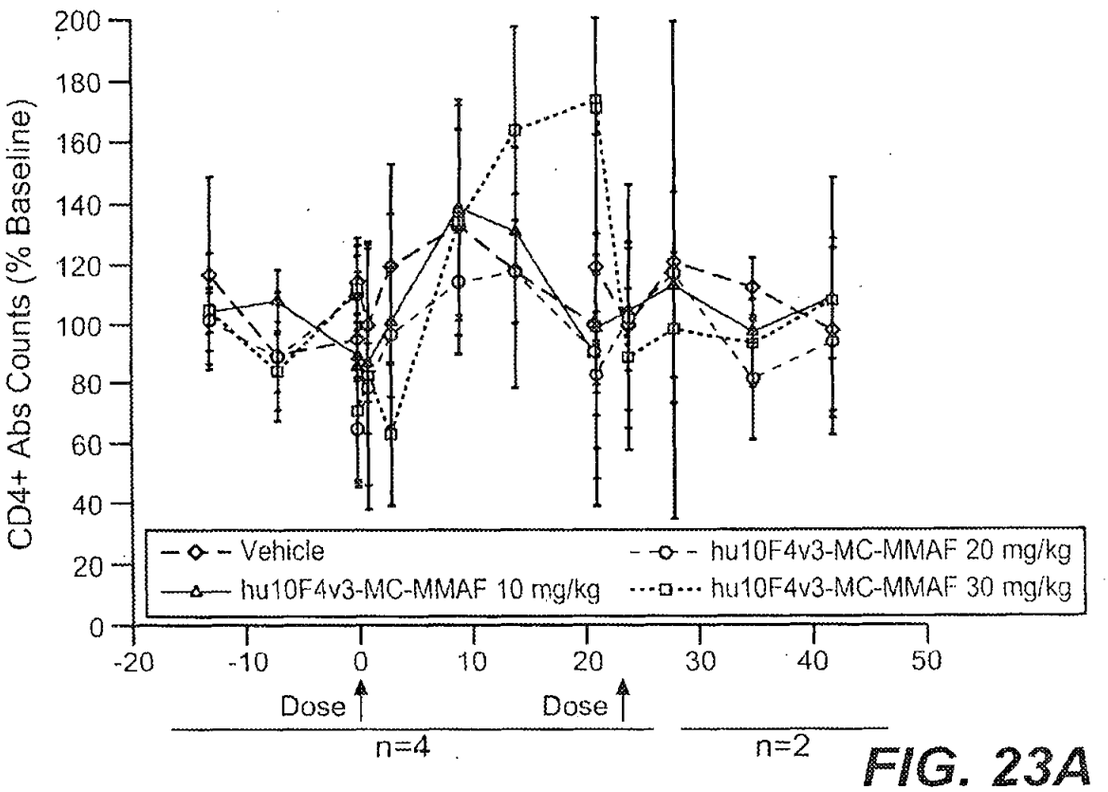
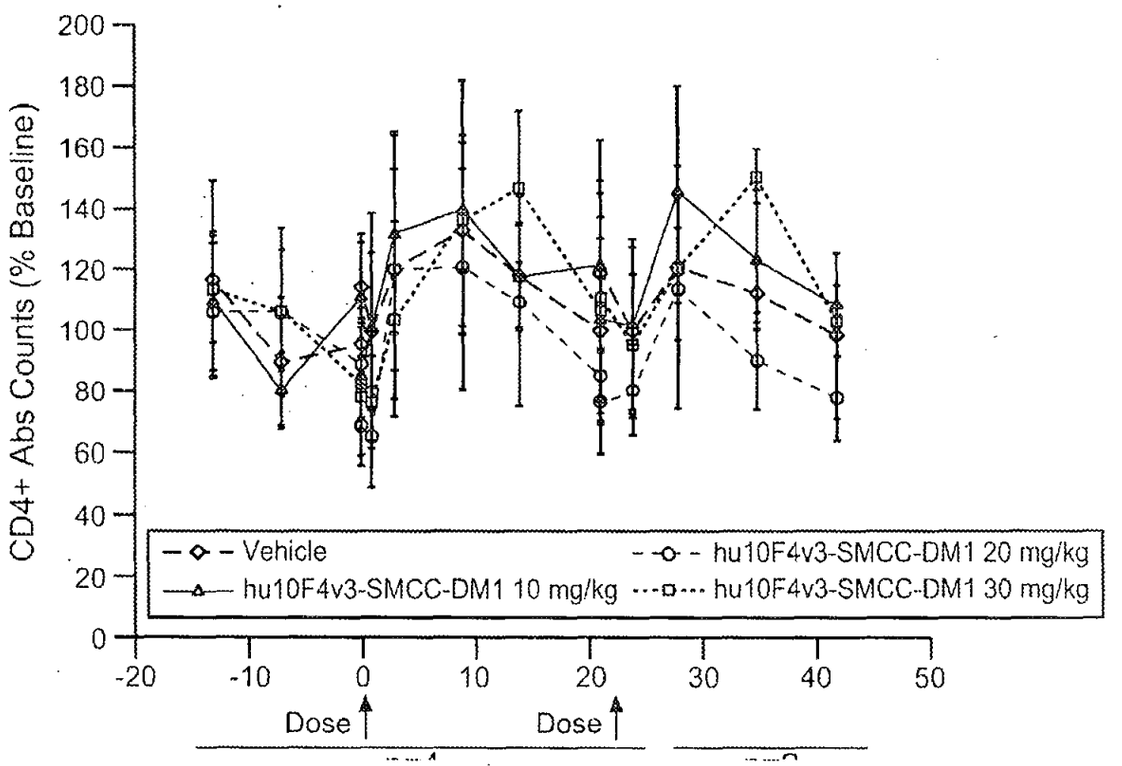

CD4+ Abs Counts (% Baseline)
200
180
160
140
120
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
Vehicle
hu10F4v3-MC-MMAF 10 mg/kg
hu10F4v3-MC-MMAF 20 mg/kg
hu10F4v3-MC-MMAF 30 mg/kg
Dose
Dose
n=4
n=2

FIG. 23A


CD4+ Abs Counts (% Baseline)
200
180
160
140
120
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
Vehicle
hu10F4v3-SMCC-DM1 10 mg/kg
hu10F4v3-SMCC-DM1 20 mg/kg
hu10F4v3-SMCC-DM1 30 mg/kg
Dose
Dose

Vehicle Control Tonsil

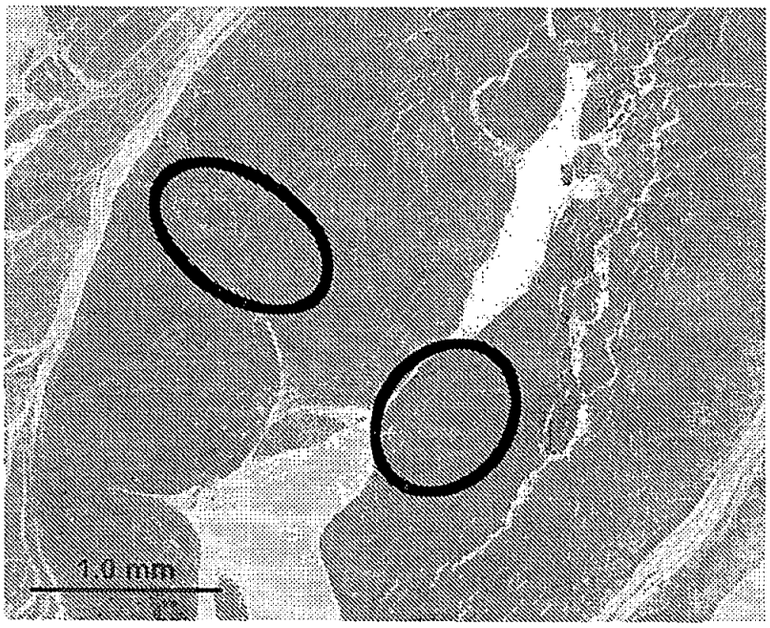


FIG. 24A

hu10F4v3-SMCC-DM1 Tonsil

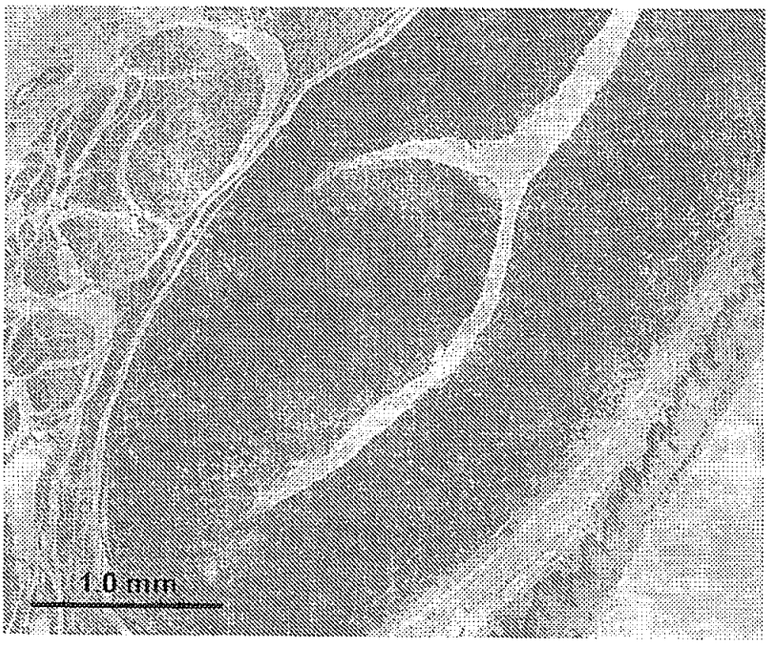
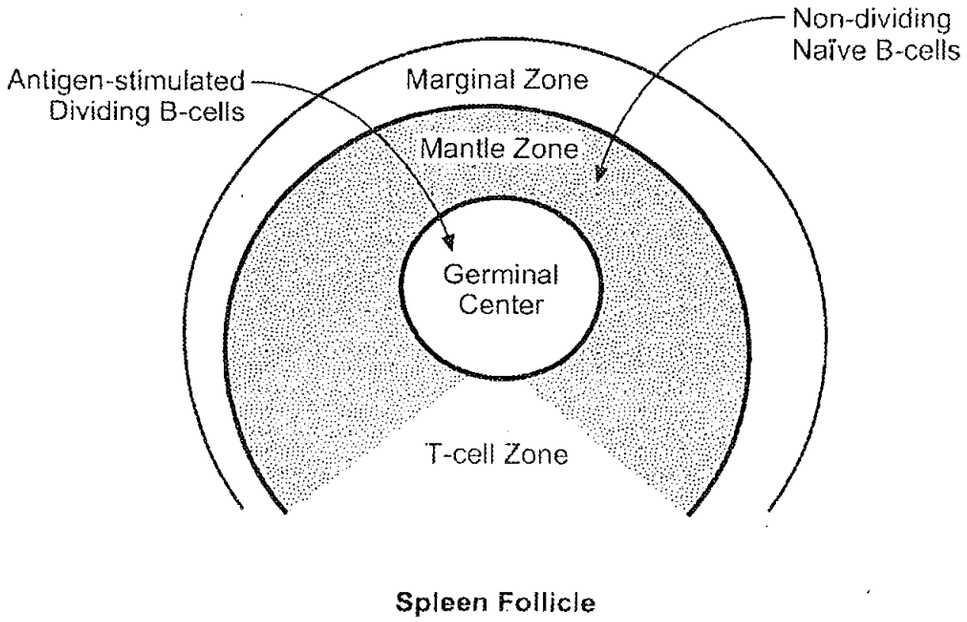


FIG. 24B

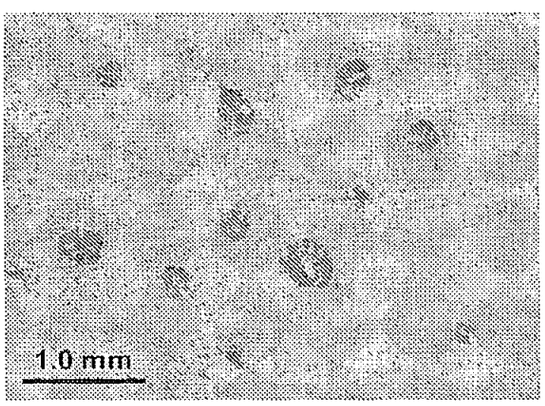


Spleen Follicle

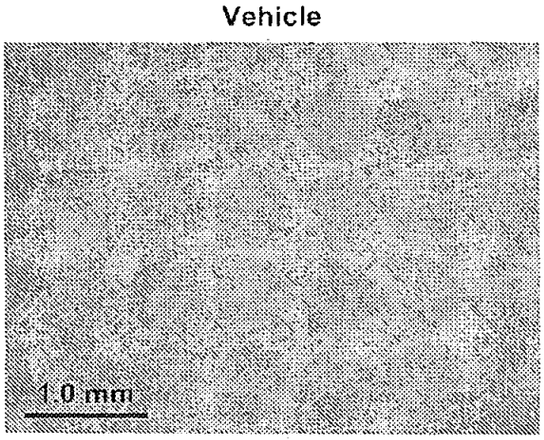
FIG. 25A

FIG. 25B

Ki-67 Staining
(Dividing Cells)



Vehicle

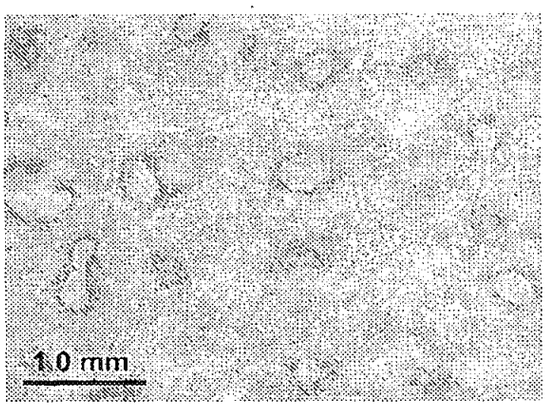


Anti-CD22-MC-MMAF
(10 mg/kg)

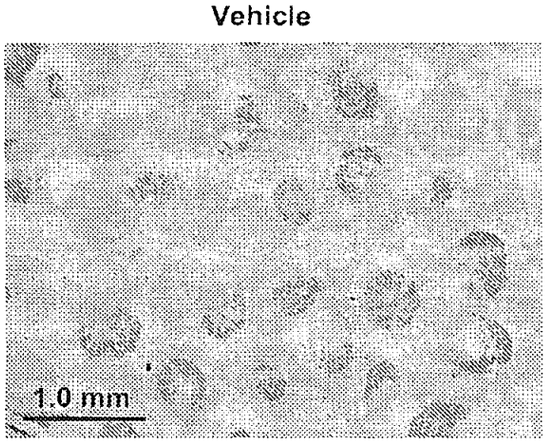
FIG. 25C

FIG. 25D

Anti-IgD Staining
(Naive B-Cells)



Vehicle



Anti-CD22-MC-MMAF
(10 mg/kg)

FIG. 25E

P2262R1SEQLISTING
Sequence Listing

<110> GENENTECH, INC. ET AL.

<120> ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREOF

<130> P2262R1

<150> US 60/911,829
<151> 2007-04-13

<150> US 60/908,941
<151> 2007-03-29

<150> US 60/809,328
<151> 2006-05-30

<160> 93

<210> 1
<211> 25
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 1
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 2
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 2
Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn
5 10

<210> 3
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 3
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
5 10

<210> 4
<211> 18
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 4
Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys
1 5 10 15

Phe Lys Gly

<210> 5
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 5
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 6
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 6
Asp Gly Ser Ser Trp Asp Trp Tyr Phe Asp Val
5 10

<210> 7
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 7
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
5 10

<210> 8
<211> 23
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 8
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys

<210> 9
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 9
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 10
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 10
Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 11
<211> 15
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 11
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 12
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 12
Lys Val Ser Asn Arg Phe Ser
5

<210> 13
<211> 32
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 13
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
20 25 30

Tyr Cys

<210> 14
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 14
Phe Gln Gly Ser Gln Phe Pro Tyr Thr
5

<210> 15
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 15
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
5 10

<210> 16
<211> 120
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp

95 100 105

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

<210> 17
<211> 113
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 17
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg
110

<210> 18
<211> 113
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr

80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg
110

<210> 19
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 19
Arg Ser Ser Gln Ser Ile Val His Ser Ala Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 20
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 20
Arg Ser Ser Gln Ser Ile Val His Ser Gln Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 21
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 21
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 22
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 22
Arg Ser Ser Gln Ser Ile Val His Ser Asp Gly Asn Thr Phe Leu

1 5 10 15

Glu

<210> 23
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 23
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 24
<211> 113
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 24
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Phe Asp Tyr Trp Gly Gln
95 100 105

Gly Thr Leu Val Thr Val Ser Ser
110

<210> 25
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val

1 5 10 15

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

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

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

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 26
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 26
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

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

<210> 27
<211> 847
<212> PRT
<213> Homo sapiens

<400> 27
Met His Leu Leu Gly Pro Trp Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr
35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser

95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val
245 250 255

Arg Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser
260 265 270

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

Leu Lys Lys Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr
290 295 300

Lys Asp Gln Ser Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val
305 310 315

Gly Pro Gly Arg Ser Glu Glu Val Phe Leu Gln Val Gln Tyr Ala
320 325 330

Pro Glu Pro Ser Thr Val Gln Ile Leu His Ser Pro Ala Val Glu
335 340 345

Gly Ser Gln Val Glu Phe Leu Cys Met Ser Leu Ala Asn Pro Leu
350 355 360

Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Met Gln Gly
365 370 375

Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu Pro Trp His
380 385 390

Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Thr Gly
395 400 405

Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro Lys

410 415 420

Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly
425 430 435

Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser
440 445 450

Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro
455 460 465

Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
470 475 480

Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser
485 490 495

Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val
500 505 510

Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val
515 520 525

Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln
530 535 540

Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln
545 550 555

Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser
560 565 570

Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp
575 580 585

Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met
590 595 600

Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr
605 610 615

Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe
620 625 630

Asp Trp Asn Asn Gln Ser Leu Pro His His Ser Gln Lys Leu Arg
635 640 645

Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln
650 655 660

Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu
665 670 675

Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val
680 685 690

Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys Gly
695 700 705

Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
710 715 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys

725 730 735

Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly
740 745 750

Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu
755 760 765

Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser
770 775 780

Ser Glu Met Gln Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr
785 790 795

Tyr Ser Ala Leu His Lys Arg Gln Val Gly Asp Tyr Glu Asn Val
800 805 810

Ile Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu
815 820 825

Ile Gln Phe Gly Val Gly Glu Arg Pro Gln Ala Gln Glu Asn Val
830 835 840

Asp Tyr Val Ile Leu Lys His
845

<210> 28
<211> 660
<212> PRT
<213> Homo sapiens

<400> 28
Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu Tyr Ala Trp Glu
1 5 10 15

Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala Leu Asp Gly
20 25 30

Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr Asn Lys
35 40 45

Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr Lys
50 55 60

Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
65 70 75

Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu
80 85 90

Asn Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu
95 100 105

Lys Trp Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe
110 115 120

Pro Pro His Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu
125 130 135

Val Thr Leu Thr Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro
140 145 150

Ile Gln Leu Gln Trp Leu Leu Glu Gly Val Pro Met Arg Gln Ala

155 160 165

Ala Val Thr Ser Thr Ser Leu Thr Ile Lys Ser Val Phe Thr Arg
170 175 180

Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser His His Gly Lys Ile
185 190 195

Val Thr Cys Gln Leu Gln Asp Ala Asp Gly Lys Phe Leu Ser Asn
200 205 210

Asp Thr Val Gln Leu Asn Val Lys His Thr Pro Lys Leu Glu Ile
215 220 225

Lys Val Thr Pro Ser Asp Ala Ile Val Arg Glu Gly Asp Ser Val
230 235 240

Thr Met Thr Cys Glu Val Ser Ser Ser Asn Pro Glu Tyr Thr Thr
245 250 255

Val Ser Trp Leu Lys Asp Gly Thr Ser Leu Lys Lys Gln Asn Thr
260 265 270

Phe Thr Leu Asn Leu Arg Glu Val Thr Lys Asp Gln Ser Gly Lys
275 280 285

Tyr Cys Cys Gln Val Ser Asn Asp Val Gly Pro Gly Arg Ser Glu
290 295 300

Glu Val Phe Leu Gln Val Gln Tyr Ala Pro Glu Pro Ser Thr Val
305 310 315

Gln Ile Leu His Ser Pro Ala Val Glu Gly Ser Gln Val Glu Phe
320 325 330

Leu Cys Met Ser Leu Ala Asn Pro Leu Pro Thr Asn Tyr Thr Trp
335 340 345

Tyr His Asn Gly Lys Glu Met Gln Gly Arg Thr Glu Glu Lys Val
350 355 360

His Ile Pro Lys Ile Leu Pro Trp His Ala Gly Thr Tyr Ser Cys
365 370 375

Val Ala Glu Asn Ile Leu Gly Thr Gly Gln Arg Gly Pro Gly Ala
380 385 390

Glu Leu Asp Val Gln Tyr Pro Pro Lys Lys Val Thr Thr Val Ile
395 400 405

Gln Asn Pro Met Pro Ile Arg Glu Gly Asp Thr Val Thr Leu Ser
410 415 420

Cys Asn Tyr Asn Ser Ser Asn Pro Ser Val Thr Arg Tyr Glu Trp
425 430 435

Lys Pro His Gly Ala Trp Glu Glu Pro Ser Leu Gly Val Leu Lys
440 445 450

Ile Gln Asn Val Gly Trp Asp Asn Thr Thr Ile Ala Cys Ala Arg
455 460 465

Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro Val Ala Leu Asn Val

470 475 480

Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys Ile Lys Pro Leu
485 490 495

Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln Cys Asp Phe
500 505 510

Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp Glu Lys Asn
515 520 525

Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe Asp Ser Ile
530 535 540

Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val Asn Asn Ser
545 550 555

Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu Val Leu Tyr
560 565 570

Ala Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly Asp Gln Val
575 580 585

Met Glu Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser Asp Ala Asn
590 595 600

Pro Pro Val Ser His Tyr Thr Trp Phe Asp Trp Asn Asn Gln Ser
605 610 615

Leu Pro His His Ser Gln Lys Leu Arg Leu Glu Pro Val Lys Val
620 625 630

Gln His Ser Gly Ala Tyr Trp Cys Gln Gly Thr Asn Ser Val Gly
635 640 645

Lys Gly Arg Ser Pro Leu Ser Thr Leu Thr Val Tyr Tyr Ser Pro
650 655 660

<210> 29
<211> 647
<212> PRT
<213> Homo sapiens

<400> 29
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr
35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser

95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Pro Pro Lys Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile
245 250 255

Arg Glu Gly Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser
260 265 270

Asn Pro Ser Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp
275 280 285

Glu Glu Pro Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp
290 295 300

Asp Asn Thr Thr Ile Ala Cys Ala Ala Cys Asn Ser Trp Cys Ser
305 310 315

Trp Ala Ser Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp
320 325 330

Val Arg Val Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly
335 340 345

Asn Ser Val Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys
350 355 360

Glu Val Gln Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys
365 370 375

Glu Ser Gln Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly
380 385 390

Ser Tyr Ser Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser
395 400 405

Lys Ala Trp Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg

410 415 420

Val Ser Met Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala
425 430 435

Thr Leu Thr Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr
440 445 450

Thr Trp Phe Asp Trp Asn Asn Gln Ser Leu Pro Tyr His Ser Gln
455 460 465

Lys Leu Arg Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr
470 475 480

Trp Cys Gln Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu
485 490 495

Ser Thr Leu Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg
500 505 510

Val Ala Val Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala
515 520 525

Ile Cys Gly Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser
530 535 540

Gln Gln Gly Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val
545 550 555

Arg Asn Lys Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His
560 565 570

Ser Leu Gly Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr
575 580 585

Thr Thr Leu Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp
590 595 600

Ala Glu Ser Ser Glu Met Gln Arg Pro Pro Pro Asp Cys Asp Asp
605 610 615

Thr Val Thr Tyr Ser Ala Leu His Lys Arg Gln Val Gly Thr Met
620 625 630

Arg Thr Ser Phe Gln Ile Phe Gln Lys Met Arg Gly Phe Ile Thr
635 640 645

Gln Ser

<210> 30
<211> 483
<212> PRT
<213> Homo sapiens

<400> 30
Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu Tyr Ala Trp Glu
1 5 10 15

Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala Leu Asp Gly
20 25 30

Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr Asn Lys

35 40 45

Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr Lys
50 55 60

Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
65 70 75

Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu
80 85 90

Asn Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu
95 100 105

Lys Trp Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe
110 115 120

Pro Pro His Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu
125 130 135

Val Thr Leu Thr Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro
140 145 150

Ile Gln Leu Gln Trp Leu Leu Glu Gly Val Pro Met Arg Gln Ala
155 160 165

Ala Val Thr Ser Thr Ser Leu Thr Ile Lys Ser Val Phe Thr Arg
170 175 180

Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser His His Gly Lys Ile
185 190 195

Val Thr Cys Gln Leu Gln Asp Ala Asp Gly Lys Phe Leu Ser Asn
200 205 210

Asp Thr Val Gln Leu Asn Val Lys His Pro Pro Lys Lys Val Thr
215 220 225

Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly Asp Thr Val
230 235 240

Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser Val Thr Arg
245 250 255

Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro Ser Leu Gly
260 265 270

Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr Thr Ile Ala
275 280 285

Cys Ala Ala Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro Val Ala
290 295 300

Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys Ile
305 310 315

Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln
320 325 330

Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp
335 340 345

Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe

350 355 360

Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val
365 370 375

Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu
380 385 390

Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly
395 400 405

Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser
410 415 420

Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe Asp Trp Asn
425 430 435

Asn Gln Ser Leu Pro Tyr His Ser Gln Lys Leu Arg Leu Glu Pro
440 445 450

Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln Gly Thr Asn
455 460 465

Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu Thr Val Tyr
470 475 480

Tyr Ser Pro

<210> 31
<211> 846
<212> PRT
<213> Pan troglodytes

<400> 31
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Glu Tyr Leu Ala
1 5 10 15

Phe Ser Asp Ser Ser Lys Trp Asn Ile Glu His Pro Gly Thr Ile
20 25 30

Tyr Ala Trp Glu Gly Ala Cys Ile Trp Val Pro Cys Thr Tyr Arg
35 40 45

Val Leu Asp Gly Ala Leu Glu Thr Phe Ile Leu Phe His Asn Pro
50 55 60

Glu Tyr Asn Gln Asn Met Ser Lys Phe Glu Gly Thr Arg Leu Tyr
65 70 75

Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Gly Gln Lys Arg Val
80 85 90

Gln Phe Leu Gly Asn Lys Ile Asn Asn Asn Cys Thr Leu Ser Ile
95 100 105

His Pro Val His Val Asn Asp Ser Gly Gln Leu Gly Leu Arg Met
110 115 120

Val Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn Val
125 130 135

Ser Glu Arg Pro Phe Pro Pro Arg Ile Gln Leu Pro Pro Lys Leu

140 145 150

Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe Ser
155 160 165

Cys Tyr Gly Tyr Gln Ile Gln Leu Gln Trp Leu Leu Glu Gly Ala
170 175 180

Pro Met Arg Gln Ala Ala Val Thr Leu Thr Ser Leu Ser Thr Lys
185 190 195

Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser
200 205 210

His His Gly Lys Ile Val Thr Cys Glu Leu His Asp Val Asp Gly
215 220 225

Lys Val Leu Ser Glu Asp Thr Val Gln Leu Asn Val Lys His Thr
230 235 240

Pro Lys Leu Thr Ile Glu Val Thr Pro Asn Glu Thr Ile Val Arg
245 250 255

Lys Gly Asp Ser Val Thr Met Thr Cys Lys Val Asn Ser Ser Asn
260 265 270

Pro Glu Tyr Thr Thr Val Ser Trp Leu Lys Asp Asp Ile Pro Leu
275 280 285

Lys Glu Gln Asn Thr Leu Met Leu Thr Leu His Glu Val Thr Lys
290 295 300

Ser Gln Thr Gly Thr Tyr Cys Cys Arg Val Ser Asn Asp Val Gly
305 310 315

Pro Ala Thr Ser Glu Lys Val Phe Leu Gln Val Gln Tyr Ala Pro
320 325 330

Glu Pro Ser Arg Val Gln Ile Ser Gln Ser Pro Ala Val Glu Gly
335 340 345

Ser Glu Val Asn Phe Leu Cys Ile Ser Pro Ala Asn Pro Leu Pro
350 355 360

Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Val Gln Gly Arg
365 370 375

Thr Glu Lys Gln Phe Gln Ile Gln Lys Ile Leu Pro Trp His Ala
380 385 390

Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Ile Gly Glu
395 400 405

Arg Gly Pro Gly Thr Glu Leu Asp Val Gln Tyr Pro Pro Lys Lys
410 415 420

Val Thr Met Val Ile Glu Asn Pro Thr Pro Ile Arg Glu Gly Asp
425 430 435

Thr Val Thr Leu Ser Cys Asn Tyr Ser Ser Ser Asn Pro Ile Val
440 445 450

Asn His Tyr Glu Trp Arg Pro Arg Gly Ala Trp Glu Glu Pro Ser

455 460 465

Leu Gly Val Leu Lys Ile Gln Asn Ile Gly Trp Asn Asn Thr Ala
470 475 480

Val Ala Cys Ala Ala Cys Asn Asn Trp Cys Ser Trp Ala Ser Pro
485 490 495

Val Thr Leu Asn Val Leu Tyr Ala Pro Arg Gly Val Arg Val Arg
500 505 510

Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Leu Val Ser
515 520 525

Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe
530 535 540

Phe Trp Glu Lys Asn Gly Ser Leu Leu Gly Lys Glu Ser Gln Leu
545 550 555

Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys
560 565 570

Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr
575 580 585

Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met Ser
590 595 600

Gln Gly Asn Gln Val Met Glu Gly Lys Thr Ala Ile Leu Thr Cys
605 610 615

Glu Ser Asp Ala Asn Pro Pro Val Tyr Ser Tyr Ala Trp Phe Asp
620 625 630

Trp Asn Asn Gln Ser Leu Pro Tyr Ser Gly Arg Met Leu Arg Leu
635 640 645

Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln Gly
650 655 660

Thr Asn Arg Val Gly Lys Gly His Ser Pro Leu Ile Thr Leu Thr
665 670 675

Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val Gly
680 685 690

Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Met Cys Gly Phe
695 700 705

Lys Val Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly Leu
710 715 720

Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys
725 730 735

Val Arg Arg Thr Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys
740 745 750

Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Ala Thr Leu Arg
755 760 765

Phe Pro Glu Thr Asn Thr Pro Arg Thr Gly Asp Ala Glu Thr Ser

770 775 780

Glu Leu Gln Arg Leu Pro Pro Asp Cys Asp Asp Thr Val Thr Tyr
785 790 795

Ser Val Leu Gln Lys Arg Gln Val Gly Asp Tyr Glu Asn Val Ile
800 805 810

Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu Ile
815 820 825

Gln Phe Gly Phe Gly Glu Arg Pro Gln Ala Gln Glu Asn Val Asp
830 835 840

Tyr Val Ile Val Lys His
845

<210> 32
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 32
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Ala Thr Phe Leu
1 5 10 15

Glu

<210> 33
<211> 16
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 33
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Gln Thr Phe Leu
1 5 10 15

Glu

<210> 34
<211> 120
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 34
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

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

Arg Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Arg

35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

<210> 35
<211> 112
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 35
Asp Ile Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
1 5 10 15

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Leu Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gly
95 100 105

Gly Thr Lys Val Glu Ile Lys
110

<210> 36
<211> 847
<212> PRT
<213> Homo sapiens

<400> 36
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr

35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser
95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val
245 250 255

Arg Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser
260 265 270

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

Leu Lys Lys Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr
290 295 300

Lys Asp Gln Ser Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val
305 310 315

Gly Pro Gly Arg Ser Glu Glu Val Phe Leu Gln Val Gln Tyr Ala
320 325 330

Pro Glu Pro Ser Thr Val Gln Ile Leu His Ser Pro Ala Val Glu
335 340 345

Gly Ser Gln Val Glu Phe Leu Cys Met Ser Leu Ala Asn Pro Leu

350 355 360

Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Met Gln Gly
365 370 375

Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu Pro Trp His
380 385 390

Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Thr Gly
395 400 405

Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro Lys
410 415 420

Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly
425 430 435

Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser
440 445 450

Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro
455 460 465

Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
470 475 480

Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser
485 490 495

Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val
500 505 510

Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val
515 520 525

Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln
530 535 540

Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln
545 550 555

Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser
560 565 570

Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp
575 580 585

Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met
590 595 600

Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr
605 610 615

Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe
620 625 630

Asp Trp Asn Asn Gln Ser Leu Pro His His Ser Gln Lys Leu Arg
635 640 645

Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln
650 655 660

Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu

665 670 675

Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val
680 685 690

Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys Gly
695 700 705

Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
710 715 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys
725 730 735

Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly
740 745 750

Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu
755 760 765

Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser
770 775 780

Ser Glu Met Gln Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr
785 790 795

Tyr Ser Ala Leu His Lys Arg Gln Val Gly Asp Tyr Glu Asn Val
800 805 810

Ile Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu
815 820 825

Ile Gln Phe Gly Val Gly Glu Arg Pro Gln Ala Gln Glu Asn Val
830 835 840

Asp Tyr Val Ile Leu Lys His
845

<210> 37
<211> 42
<212> PRT
<213> Homo sapiens

<400> 37
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
1 5 10 15

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

Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys
35 40

<210> 38
<211> 218
<212> PRT
<213> Homo sapiens

<400> 38
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val

20 25 30

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
35 40 45

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
50 55 60

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
65 70 75

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
80 85 90

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
95 100 105

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
110 115 120

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
125 130 135

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
140 145 150

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
155 160 165

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
170 175 180

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
185 190 195

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
200 205 210

Ser Leu Ser Leu Ser Pro Gly Lys
215

<210> 39
<211> 217
<212> PRT
<213> Homo sapiens

<400> 39
Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
1 5 10 15

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
20 25 30

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
35 40 45

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
50 55 60

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
65 70 75

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

80 85 90

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr
95 100 105

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
110 115 120

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
125 130 135

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
140 145 150

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
155 160 165

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
170 175 180

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
185 190 195

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
200 205 210

Leu Ser Leu Ser Pro Gly Lys
215

<210> 40
<211> 217
<212> PRT
<213> Homo sapiens

<400> 40
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
35 40 45

Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
50 55 60

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
65 70 75

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
80 85 90

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
95 100 105

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
110 115 120

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
125 130 135

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

140 145 150

Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro
155 160 165

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
170 175 180

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys
185 190 195

Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser
200 205 210

Leu Ser Leu Ser Pro Gly Lys
215

<210> 41
<211> 218
<212> PRT
<213> Homo sapiens

<400> 41
Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
35 40 45

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
50 55 60

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
65 70 75

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
80 85 90

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
95 100 105

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
110 115 120

Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
125 130 135

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
140 145 150

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
155 160 165

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg
170 175 180

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
185 190 195

Cys Ser Val Met His Glu Ala Leu His Asn His Thr Tyr Gln Lys

200 205 210

Ser Leu Ser Leu Ser Leu Gly Lys
215

<210> 42
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 42
Cys Asp Lys Thr His Thr Gly Gly Gly Ser Gln Arg Leu Met Glu
1 5 10 15

Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp Asp Phe
20 25 30

<210> 43
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 43
Gln Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu
1 5 10 15

Trp Glu Asp Asp Phe
20

<210> 44
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 44
Gln Arg Leu Ile Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu
1 5 10 15

Trp Glu Asp Asp Phe
20

<210> 45
<211> 18
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 45
Arg Leu Ile Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1 5 10 15

Glu Asp Asp

<210> 46
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 46
Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
5 10

<210> 47
<211> 14
<212> PRT
<213> Homo sapiens

<400> 47
Trp Val Arg Arg Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
5 10

<210> 48
<211> 32
<212> PRT
<213> Homo sapiens

<400> 48
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

Ala Arg

<210> 49
<211> 23
<212> PRT
<213> Homo sapiens

<400> 49
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro
1 5 10 15

Gly Glu Pro Ala Ser Ile Ser Cys
20

<210> 50
<211> 25
<212> PRT
<213> Homo sapiens

<400> 50
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser
20 25

<210> 51

<211> 13
<212> PRT
<213> Homo sapiens

<400> 51
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
5 10

<210> 52
<211> 31
<212> PRT
<213> Homo sapiens

<400> 52
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

Ala

<210> 53
<211> 30
<212> PRT
<213> Homo sapiens

<400> 53
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

<210> 54
<211> 30
<212> PRT
<213> Homo sapiens

<400> 54
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15

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

<210> 55
<211> 14
<212> PRT
<213> Homo sapiens

<400> 55
Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
5 10

<210> 56
<211> 32
<212> PRT
<213> Homo sapiens

<400> 56
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu

1 5 10 15

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

Ala Arg

<210> 57
<211> 25
<212> PRT
<213> Homo sapiens

<400> 57
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1 5 10 15

Gln Thr Leu Ser Leu Thr Cys Thr Val Ser
20 25

<210> 58
<211> 13
<212> PRT
<213> Homo sapiens

<400> 58
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5 10

<210> 59
<211> 31
<212> PRT
<213> Homo sapiens

<400> 59
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
1 5 10 15

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

Ala

<210> 60
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<213> Homo sapiens

<400> 60
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1 5 10 15

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

<210> 61
<211> 30
<212> PRT
<213> Homo sapiens

<400> 61
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 62
<211> 14
<212> PRT
<213> Homo sapiens

<400> 62
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<210> 63
<211> 32
<212> PRT
<213> Homo sapiens

<400> 63
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala Arg

<210> 64
<211> 25
<212> PRT
<213> Homo sapiens

<400> 64
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 65
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<400> 65
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
5 10

<210> 66
<211> 31
<212> PRT
<213> Homo sapiens

<400> 66
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala

<210> 67
<211> 30
<212> PRT
<213> Homo sapiens

<400> 67
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 68
<211> 30
<212> PRT
<213> Homo sapiens

<400> 68
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

<210> 69
<211> 32
<212> PRT
<213> Homo sapiens

<400> 69
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ser Arg

<210> 70
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Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ser

<210> 71
<211> 32
<212> PRT
<213> Homo sapiens

<400> 71
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1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala Arg

<210> 72
<211> 31
<212> PRT
<213> Homo sapiens

<400> 72
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala

<210> 73
<211> 30
<212> PRT
<213> Homo sapiens

<400> 73
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 74
<211> 23
<212> PRT
<213> Homo sapiens

<400> 74
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Gly Asp Arg Val Thr Ile Thr Cys
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<210> 75
<211> 15
<212> PRT
<213> Homo sapiens

<400> 75
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 76
<211> 32
<212> PRT
<213> Homo sapiens

<400> 76
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe

1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
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Tyr Cys

<210> 77
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Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
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Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
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Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
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<210> 80
<211> 32
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<400> 80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

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

Tyr Cys

<210> 81
<211> 23
<212> PRT
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1 5 10 15

Gly Glu Arg Ala Thr Leu Ser Cys
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<210> 82

<211> 15
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<213> Homo sapiens

<400> 82
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 83
<211> 32
<212> PRT
<213> Homo sapiens

<400> 83
Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr
20 25 30

Tyr Cys

<210> 84
<211> 23
<212> PRT
<213> Homo sapiens

<400> 84
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
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Gly Glu Arg Ala Thr Ile Asn Cys
20

<210> 85
<211> 15
<212> PRT
<213> Homo sapiens

<400> 85
Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 86
<211> 32
<212> PRT
<213> Homo sapiens

<400> 86
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr
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Tyr Cys

<210> 87
<211> 219
<212> PRT
<213> Artificial sequence

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<400> 87
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
110 115 120

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
125 130 135

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
140 145 150

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
155 160 165

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
170 175 180

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
185 190 195

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
200 205 210

Thr Lys Ser Phe Asn Arg Gly Glu Cys
215

<210> 88
<211> 450
<212> PRT
<213> Artificial sequence

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<223> sequence is synthesized

<400> 88
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Glu Phe Ser

20 25 30

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
320 325 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln

335 340 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

<210> 89
<211> 120
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

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Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
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Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Glu Phe Ser
20 25 30

Arg Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Arg
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

<210> 90
<211> 112
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

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1 5 10 15

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Leu Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val
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Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gly
95 100 105

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<210> 91
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<213> Artificial sequence

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

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
110 115 120

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
125 130 135

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

140 145 150

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
155 160 165

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
170 175 180

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
185 190 195

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Cys
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Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<210> 92
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<223> sequence is synthesized

<400> 92
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Glu Phe Ser
20 25 30

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Cys Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser

185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
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Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
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350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

<210> 93
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<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

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

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala

320 325 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
335 340 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Cys Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

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(84) Países designados *(a menos que se indique lo contrario para cada clase de protección disponible)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Título: ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

(57) Extracto: Se proporcionan anticuerpos anti-CD22 y sus inmunoconjugados. Se proporcionan métodos de uso de los anticuerpos anti-CD22 y de sus inmunoconjugados.

Expediente de patente N.°: **P2262R1**

ANTICUERPOS E INMUNOCONJUGADOS Y SUS USOS

CAMPO DE LA INVENCIÓN

La presente invención se relaciona con los anticuerpos anti-CD22 y sus inmunoconjugados. La invención se refiere además, a los métodos de utilización de los anticuerpos anti-CD22 y de sus inmunoconjugados.

ANTECEDENTES

Los linfocitos son una de las muchas clases de glóbulos blancos que se producen en la médula ósea durante la hematopoyesis. Existen dos poblaciones principales de linfocitos: los linfocitos B (células B) y T (células T). Los linfocitos que nos interesan de manera particular en este estudio son las células B.

Las células B maduran en la médula ósea y la abandonan expresando un anticuerpo de unión al antígeno en su superficie celular. Cuando una célula B virgen se encuentra con el antígeno para el que su anticuerpo unido a la membrana es específico, la célula comienza a dividirse rápidamente y las células resultantes se diferencian en células B de memoria y células efectoras denominadas "células plasmáticas". Las células B de memoria tienen mayor duración y continúan expresando el anticuerpo unido a la membrana con la misma especificidad que la célula parental original. Las células plasmáticas no producen un anticuerpo unido a la membrana, sino un anticuerpo en forma secretada. Los anticuerpos secretados son las principales moléculas efectoras de la inmunidad humoral.

Los trastornos relacionados con las células B incluyen, entre otros, linfomas cancerosos (linfoma no de Hodgkin, LNH), mielomas múltiples y leucemia linfocítica crónica (LLC, leucemia de células B (linfocitos B CD5+)). Los linfomas de no Hodgkin (LNH), un grupo heterogéneo de cánceres que generan principalmente los linfocitos B, representan aproximadamente un 4% de todos los cánceres diagnosticados recientemente (Jemal, A. y col., *CA-Cancer J Clin, 52:* 23-47, (2002)). El LNH agresivo comprende aproximadamente entre el 30 y 40% de los LNH en adultos (Harris, N.L. y col., *Hematol. J. 1:*53-66 (2001)) e incluye un linfoma de células B grandes difuso (LCBGD), un linfoma de células del manto (LCM), un linfoma T periférico y un linfoma anaplásico de células grandes. La quimioterapia de combinación de primera línea cura a menos de la mitad de los pacientes con LNH agresivo y la mayoría de éstos sucumben a la enfermedad (Fisher, R.I. Semin. Oncol. 27(suppl 12): 2-8 (2000)).

Los trastornos relacionados con las células B también incluyen enfermedades autoinmunes. Las enfermedades autoinmunes siguen siendo importantes desde un punto de vista clínico en humanos. Como su nombre indica, las enfermedades autoinmunes actúan sobre el propio sistema inmunológico del organismo. Si bien los mecanismos patológicos difieren entre los distintos tipos de enfermedades autoinmunes, un mecanismo general supone la unión de determinados anticuerpos (denominados en adelante anticuerpos autorreactivos o autoanticuerpos) a las proteínas endógenas del organismo. Médicos y científicos han identificado más de 70 enfermedades autoinmunes clínicamente diferentes, incluidas artritis reumatoide, esclerosis múltiple, vasculitis, diabetes mediada por la autoinmunidad y lupus eritematoso sistémico. Aunque muchas enfermedades autoinmunes son poco frecuentes (afectan a menos de 200.000 sujetos cada una), en conjunto, padecen estas enfermedades millones de estadounidenses, que representan a un 5% de la población, y la mayoría de estas enfermedades afectan a las mujeres mucho más que a los hombres. El carácter crónico de estas enfermedades ocasiona una carga económica y social inmensa.

Los agentes citotóxicos que actúan sobre los antígenos de la superficie de las células B constituyen un enfoque importante de los tratamientos para el cáncer relacionado con dichas células. Uno de estos antígenos de la superficie de las células B es el CD20. El rituximab (Rituxan; Genentech, Inc. (sur de San Francisco, CA) y IDEC Pharmaceutical Corp. (San Diego, CA)), un anticuerpo monoclonal quimérico anti-CD20 (ratón/humano), fue el primer anticuerpo terapéutico que aprobó la Administración de Alimentos y Fármacos estadounidense para el tratamiento de LNH folicular o de bajo grado recurrente o refractario (Leonard, J.P. y col., *Clin. Canc. Res.* 10:5327-5334 (2004)).

Otros antígenos de células B, como por ejemplo CD19, CD22 y CD52, son dianas de potencial terapéutico para tratar el linfoma (Grillo-Lopez A.J. y col., *Curr Pharm Biotechnol*, 2:301-11, (2001)). CD22 es una sialoglicoproteína restringida a células B 135-kDa que sólo se expresa en la superficie de las células B durante las etapas de maduración de diferenciación (Dorken, B. y col., *J. Immunol.* 136:4470-4479 (1986)). La forma predominante de CD22 en los humanos es CD22 beta que contiene siete dominios de la superfamilia de las inmunoglobulinas en el dominio extracelular (Figura 1) (Wilson, G.L. y col., *J. Exp. Med.* 173:137-146 (1991)). Una variante de esta forma, CD22 alfa, no contiene los dominios 3 y 4 de la superfamilia de las inmunoglobulinas (Stamenkovic, I. y Seed, B., *Nature* 345:74-77 (1990)). Se ha demostrado que la unión del ligando al CD22 humano se relaciona con los dominios 1 y 2 de la superfamilia de las inmunoglobulinas (también conocidos como epítopos 1 y 2) (Engel, P. y col., *J. Exp. Med.* 181:1581-1586, 1995).

En el LNH de células B, la expresión de CD22 oscila entre el 91 y 99% en poblaciones agresivas e indolentes, respectivamente (Cesano, A. y col., *Blood* 100:350a (2002)). El CD22 puede funcionar como

un componente del complejo de activación de las células B (Sato, S. y col., *Semin. Immunol.* 10:287-296 (1998)) y como una molécula de adhesión (Engel, Pl y col., J. *Immunol.* 150:4719-4732 (1993)). Las células B de los ratones con deficiencia de CD22 tienen una menor duración y una apoptosis mejorada que sugiere un papel clave de este antígeno en la supervivencia de la célula B (Otipoby, K.L. y col., *Nature (Lond)* 384:634-637 (1996)). Después de unirse a los ligandos naturales o anticuerpos, el CD22 se internaliza rápidamente y proporciona una señal coestimuladora fuerte en las células B primarias y señales proapoptóticas en las células B neoplásicas (Sato, S. y col., *Immunity* 5:551-562 (1996)).

Se han estudiado los anticuerpos anti-CD22 como posibles tratamientos para los cánceres de células B y otras enfermedades proliferativas de las células B. Estos anticuerpos anti-CD22 incluyen el RFB4 (Mansfield, E. y col., *Blood* 90:2020-2026 (1997)), el CMC-544 (DiJoseph, J.F., *Blood* 103:1807-1814 (2004)) y el LL2 (Pawlak-Byczkowska, E.J. y col., *Cancer Res.* 49:4568-4577 (1989)). El anticuerpo LL2 (anteriormente denominado HPB-2) es un anticuerpo monoclonal de ratón IgG2a que se dirige contra el antígeno CD22 (Pawlak-Byczkowska, E.J. y col. (1989), mencionado anteriormente). Las evaluaciones inmunohistológicas in vitro demostraron una reactividad del anticuerpo LL2 con 50 de los 51 especímenes del LNH de células B evaluados. Sin embargo, esto no ocurrió con otros tejidos no linfoides normales o malignos (Pawlak-Byczkowska (1989), mencionado anteriormente; Stein, R. y col., *Cancer Immunol. Immunother.* 37:293-298 (1993)).

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 diferia, 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.° 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.

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.° 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). 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".

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.

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 I 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).

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).

Los anticuerpos anti-CD22 conjugados con toxinas también se estudiaron como compuestos terapéuticos potenciales. Por ejemplo, estudios anteriores describen las inmunotoxinas que contienen cadenas de ricina A dirigidas contra los anti-CD22 como posibles agentes antineoplásicos (May, R.D. y col., *Chemical Abstracts* 106(21):168656x páginas 35-36 (1987); Ghetie, M.A. y col., *Cancer Research* 48:2610-2617 (1988) y Amlot, P.L. y col., *Blood* 82(9):2624-2633 (1993)). Cuando la toxina es un radioisótopo, epratuzumab, la versión del IgG1 (transplantada de CDR) humanizado de LL2, mostró evidencia de actividad terapéutica para el radioinmunoconjugado (Juweid, M.E. y col., *Clin. Cancer Res.*, 5 (Supl 10):3292s-3303s (1999); Griffiths, G.L. y col., *J. Nucl. Med.* 44:77-84 (2003); Linden, O. y col., *Clin. Cancer Res.*, 5 (supl 10):3287s-3291s (1999)).

En la materia existe la necesidad de otros fármacos para tratar diversos cánceres relacionados con las células B, como por ejemplo los linfomas como el linfoma no Hodgkin y otros trastornos proliferativos de las células B. Los fármacos especialmente útiles para este fin incluyen los conjugados de anticuerpos anti-CD22 con fármacos que actúan sobre las células B 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.

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

La invención proporciona anticuerpos anti-CD22 y sus métodos de utilización.

En un aspecto, se proporciona un anticuerpo que se une a CD22, en el que el anticuerpo comprende al menos una, dos, tres, cuatro, cinco o seis HVR selecionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;

(4) una HVR-L1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 10;

(5) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 10, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 9, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-H3 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 6, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-L1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 9;

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 10, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 9, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y

(6) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-H3 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 5, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;

(3) una HVR-L1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 9;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 12; y

(5) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-H3 que contiene la secuencia de aminoácidos de identificador de secuencia n.°: 6, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 4;

(3) una HVR-L1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 10;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 12; y

(5) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 14.

En una forma de realización, el anticuerpo comprende una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 10. En una forma de realización, el anticuerpo también comprende una HVR-H1 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 2 y una HVR-H2 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 4. En una forma de realización, el anticuerpo también comprende una HVR-L2 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 12 y una HVR-L3 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 14.

En ciertas formas de realización, cualquiera de los anticuerpos anteriores comprende, además, al menos una estructura seleccionada entre una estructura de consenso del subgrupo III de VH y una estructura de consenso del sugrupo I de VL.

En un aspecto, se proporciona un anticuerpo que se une a CD22, 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.° 16. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena pesada de identificador de secuencia n.° 16.

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 12; y

(5) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 14.

En otro aspecto, un anticuerpo que se une a CD22 comprende (a) una HVR-H3 que contiene la secuencia de aminoácidos de identificador de secuencia n.°: 5, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:

(1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 2;

(2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 4;

(3) una HVR-L1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 10;

(4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 12; y

(5) una HVR-L3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.°: 14.

En una forma de realización, el anticuerpo comprende una HVR-L1 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 10. En una forma de realización, el anticuerpo también comprende una HVR-H1 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 2 y una HVR-H2 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 4. En una forma de realización, el anticuerpo también comprende una HVR-L2 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 12 y una HVR-L3 que contiene la secuencia de aminoácidos de identificador de secuencia n.° 14.

En ciertas formas de realización, cualquiera de los anticuerpos anteriores comprende, además, al menos una estructura seleccionada entre una estructura de consenso del subgrupo III de VH y una estructura de consenso del sugrupo I de VL.

En un aspecto, se proporciona un anticuerpo que se une a CD22, 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.° 16. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena pesada de identificador de secuencia n.° 16.

HOJA MODIFICADA

En un aspecto, 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.° 17. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena ligera de identificador de secuencia n.° 17.

En un aspecto, 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.° 18. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena ligera de identificador de secuencia n.° 18.

En una forma de realización, el anticuerpo comprende un dominio variable de la cadena pesada con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 ó 100% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.° 16 y un dominio variable de la cadena ligera con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 ó 100% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.° 17. En una forma de realización, el anticuerpo comprende un dominio variable de la cadena pesada con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 ó 100% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.° 16 y un dominio variable de la cadena ligera con al menos un 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 ó 100% de identificador de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.° 18. En una forma de realización, el dominio variable de la cadena pesada comprende la secuencia de aminoácidos de identificador de secuencia n.° 16 y el dominio variable de la cadena ligera comprende la secuencia de aminoácidos de identificador de secuencia n.° 17. En una forma de realización, el dominio variable de la cadena pesada comprende la secuencia de aminoácidos de identificador de secuencia n.° 16 y el dominio variable de la cadena ligera comprende la secuencia de aminoácidos de identificador de secuencia n.° 18.

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-CD22, 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.

En un aspecto, se proporciona un anticuerpo que se une a CD22 expresado en la superficie de una célula. En una forma de realización, el anticuerpo se une a un epítopo en la región del CD22 humano o de ratón que comprende el dominio 1 ó 2 o los dominios 1 y 2. 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 B. En una forma de realización, la célula cancerosa es una célula B.

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.

En una aspecto, se proporciona un método de detección de la presencia de CD22 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 CD22 y detectar si un complejo está formado por el anticuerpo y CD22. En una forma de realización, la muestra biológica comprende las células B. En una forma de realización, la muestra biológica se obtiene de un mamífero que experimenta o se cree que experimenta un trastorno de células B o un trastorno proliferativo de células B, o ambos, incluyendo, entre otros, linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

En un aspecto, se proporciona un método de diagnóstico de un trastorno de proliferación celular asociado a un aumento de la expresión de CD22. Este método comprende establecer un contacto entre una célula de prueba y cualquiera de los anticuerpos anteriores, determinar el nivel de expresión de CD22 mediante la detección de la unión del anticuerpo a CD22 y comparar el nivel de expresión de CD22 mediante la célula de prueba con el nivel de expresión de CD22 mediante una célula de control, donde un nivel más elevado de expresión de CD22 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 CD22. 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 células B. En una forma de realización, el trastorno proliferativo de células B se selecciona a partir de los trastornos de células B que incluyen, entre otros, linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma

linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. En una forma de realización, el método comprende la determinación del nivel de expresión de CD22 en la superficie de la célula de prueba y la comparación del nivel de expresión de CD22 en la superficie de la célula de prueba con el nivel de expresión de CD22 en la superficie de la célula de control.

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 B, que expresan CD22. 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 CD22 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 CD22 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 CD22.

En un aspecto, se proporciona un método de detección de CD22 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 B con un anticuerpo anti-CD22 de la invención, y detectar un aumento en el CD22 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 B asociados a un aumento de CD22 soluble en la sangre o el suero de un mamífero.

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 sin cisteína, tal como se describe en el documento WO2006/034488 (íntegramente incorporado en la presente memoria descriptiva a modo de referencia). Cualquier forma de anticuerpos anti-CD22 puede diseñarse de esta manera, es decir, puede mutarse. Por ejemplo, un fragmento Fab del anticuerpo parental puede diseñarse para formar un Fab diseñado con cisteína, denominado "ThioFab" en la presente memoria descriptiva. Asimismo, un anticuerpo monoclonal parental puede diseñarse para formar un "ThioMab". Se debe tener en cuenta que una mutación en un único sitio produce un solo residuo diseñado con cisteína en un ThioFab, mientras una mutuación en un único sitio produce dos residuos diseñados con cisteína en un ThioMab. Esto se debe a la naturaleza dimérica del anticuerpo IgG. Los anticuerpos anti-CD22 diseñados con cisteína de la invención incluyen anticuerpos monoclonales, anticuerpos monoclonales humanizados o

quiméricos, y fragmentos de unión a antígeno de los anticuerpos, polipéptidos de fusión y análogos que preferentemente unen polipéptidos CD22 asociados a las células. Un anticuerpo diseñado con cisteína puede comprender alternativamente un anticuerpo que contenga una cisteína en el anticuerpo o Fab, con una posición detallada en la presente memoria descriptiva, que se obtiene del diseño o selección, o ambos, de la secuencia del anticuerpo, sin necesariamente alterar un anticuerpo parental, como por ejemplo, mediante un diseño y selección de anticuerpos presentados en fagos o a través de un diseño nuevo de secuencias estructurales y regiones constantes de la cadena ligera o pesada, o ambas. Un anticuerpo diseñado con cisteína comprende uno o más aminoácidos sin cisteína con un valor de reactividad del tiol en un rango de entre 0,6 y 1,0; 0,7 y 1,0 ó 0,8 y 1,0. Un aminoácido sin cisteína es un residuo de cisteína que se ha diseñado como 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 cys 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).

En una forma de realización, un anticuerpo anti-CD22 de la invención diseñado con cisteína comprende una cisteína diseñada en cualquiera de las siguientes posiciones, donde la posición es el número en la cadena ligera según Kabat y col., (véase Kabat y col., (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD) y en la cadena pesada según la numeración EU (incluyendo la región Fc) (véase Kabat y col., (1991), mencionado anteriormente), en donde la región constante de la cadena ligera que se subraya en la Figura 17A comienza en la posición 108 (numeración de Kabat) y la región constante de la cadena pesada que se muestra subrayada en las Figuras 17B y 17C comienza en la posición 118 (numeración EU). La posición también puede describirse por su posición en la numeración secuencial de los aminoácidos de las cadenas ligera o pesada de longitud completa que se muestran en las Figuras 17A-17C. Según una forma de realización de la invención, un anticuerpo anti-CD22 comprende una cisteína diseñada en LC-V205C (número según Kabat: Val 205; número secuencial 210 en la Figura 17A diseñado para que sea cys en esa posición). La cisteína diseñada en la cadena ligera se muestra en un texto en negrita con doble subrayado en la Figura 17A. Según una forma de realización, un anticuerpo anti-CD22 comprende una cisteína diseñada en HC-A118C (número EU: Ala 118; número secuencial 121 en la Figura 17B diseñado para que sea cys en esa posición). La cisteína diseñada en la cadena pesada se muestra en un texto en negrita con

doble subrayado en la Figura 17B. Según una forma de realización, un anticuerpo anti-CD22 comprende una cisteína diseñada en Fc-S400C (número EU: Ser 400; número secuencial 403 en la Figura 17C diseñado para que sea cys en esa posición). La cisteína diseñada en la región Fc de la cadena pesada se muestra en un texto en negrita con doble subrayado en la Figura 17C. En otras formas de realización, la cisteína diseñada de la cadena pesada (incluyendo la región Fc) se encuentra en cualquiera de las siguientes posiciones (según la numeración EU): 41, 88, 116, 118, 120, 171, 282, 375 ó 400. Por lo tanto, los cambios en los aminoácidos en estas posiciones para un anticuerpo anti-CD22 parental de la invención son: A41C, A88C, S116C, A118C, T120C, A171C, V282C, S375C o S400C. En otras formas de realización, la cisteína diseñada de la cadena ligera se encuentra en cualquiera de las siguientes posiciones (según la numeración de Kabat): 15, 43, 110, 144, 168, 205. Por lo tanto, los cambios en los aminoácidos en estas posiciones para un anticuerpo anti-CD22 parental de la invención son: V15C, A43C, V110C, A144C, S168C o V205C.

Un anticuerpo anti-CD22 diseñado con cisteína comprende uno o más aminoácidos sin cisteína, en donde el anticuerpo anti-CD22 diseñado con cisteína se une al polipéptido CD22 y se prepara mediante un proceso que comprende el reemplazo de uno o más residuos de aminoácidos de un anticuerpo anti-CD22 parental por cisteína, en donde el anticuerpo parental comprende al menos una secuencia HVR seleccionada de:

(a) una secuencia RSSQSIVHSNGNTFLE (identificador de secuencia n.° 9) o una secuencia RSSQSIVHSVGNTFLE (identificador de secuencia n.° 10) de HVR-L1 (Figura 2B);

(b) una secuencia KVSNRFS (identificador de secuencia n.° 12) de HVR-L2 (Figura 2B);

(c) una secuencia FQGSQFPYT (identificador de secuencia n.° 14) de HVR-L3 (Figura 2B);

(d) una secuencia GYEFSRSWMN (identificador de secuencia n.° 2) de HVR-H1 (Figura 2A);

(e) una secuencia GRIYPGDGDTNYSGKFKG (identificador de secuencia n.° 4) de HVR-H2 (Figura 2A); y

(f) una secuencia DGSSWDWYFDV (identificador de secuencia n.° 6) de HVR-H3 (Figura A).

En cierto aspecto, la invención comprende un anticuerpo anti-CD22 diseñado con cisteína que contiene una secuencia de aminoácidos con al menos un 80% de identificación de secuencia de

aminoácidos, en forma alternativa con al menos un 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 ó 100% de identificación de secuencia de aminoácidos, en un anticuerpo diseñado con cisteína con una secuencia de aminoácidos de longitud completa o una secuencia de aminoácidos del anticuerpo diseñado con cisteína carente de un péptido de señal, tal como se detalla en la presente memoria descriptiva.

En un segundo aspecto adicional, la invención se refiere a un anticuerpo anti-CD22 aislado diseñado con cisteína que comprende una secuencia de aminoácidos que se codifica mediante una secuencia de nucleótidos que se hibridiza con el complemento de una molécula de ADN que codifica (a) un anticuerpo diseñado con cisteína con una secuencia de aminoácidos de longitud completa, tal como se detalla en la presente memoria descriptiva, (b) una secuencia de aminoácidos del anticuerpo diseñado con cisteína carente de un péptido de señal, tal como se detalla en la presente memoria descriptiva, (c) un dominio extracelular de una proteína transmembrana del anticuerpo diseñado con cisteína, con el péptido de señal o sin él, tal como se detalla en la presente memoria descriptiva, (d) una secuencia de aminoácidos que se codifica mediante cualquiera de las secuencias de ácidos nucleicos, tal como se detalla en la presente memoria descriptiva o (e) cualquier otro fragmento específicamente definido de una secuencia de aminoácidos del anticuerpo diseñado con cisteína de longitud completa, tal como se detalla en la presente memoria descriptiva.

En un aspecto específico, la invención proporciona un anticuerpo anti-CD22 aislado diseñado con cisteína sin la secuencia de señal N-terminal ni la metionina inicial, y se codifica mediante una secuencia de nucleótidos que codifica dicha secuencia de aminoácidos, tal como se detalla en la presente memoria descriptiva. Los procesos para producir dicho anticuerpo también se detallan en la presente memoria descriptiva, en donde los procesos comprenden el cultivo de una célula huésped que contiene un vector con la correspondiente molécula de ácido nucleico de codificación bajo las condiciones adecuadas para la expresión del anticuerpo diseñado con cisteína, y la recuperación del anticuerpo diseñado con cisteína del cultivo celular.

Otro aspecto de la invención proporciona un anticuerpo anti-CD22 aislado diseñado con cisteína que es un transmembrana con dominio eliminado o inactivo. Los procesos para producir dicho anticuerpo también se detallan en la presente memoria descriptiva, en done esos procesos comprenden el cultivo de una célula huésped con un vector que contiene la correspondiente molécula de ácido nucleico codificada bajo las condiciones adecuadas para la expresión del anticuerpo diseñado con cisteína, y la recuperación del anticuerpo diseñado con cisteína del cultivo celular.

En otras formas de realización, la invención proporciona anticuerpos anti-CD22 quiméricos aislados diseñados con cisteína que comprenden cualquiera de los anticuerpos diseñados con cisteína que se detallan en la presente memoria descriptiva, fusionados a un polipéptido heterólogo (no CD22). Un ejemplo de dichas moléculas quiméricas comprende cualquiera de los anticuerpos diseñados con cisteína que se detallan en la presente memoria descriptiva, fusionados a un polipéptido heterólogo tal como una secuencia de marca de epitopo o una región Fc de una inmunoglobulina.

El anticuerpo anti-CD22 diseñado con cisteína puede ser un anticuerpo monoclonal, un fragmento de anticuerpo, un anticuerpo quimérico, un anticuerpo humanizado, un anticuerpo de cadena única o un anticuerpo que inhiba competitivamente la unión del anticuerpo polipéptido anti-CD22 a su correspondiente epítopo antigénico. Los anticuerpos de la presente invención pueden conjugarse opcionalmente con un agente inhibidor del crecimiento o un agente citotóxico tal como una toxina, incluyendo, por ejemplo, una auristatina, un antibiótico, un isótopo radioactivo, una enzima nucleolítica o similares. Los anticuerpos de la presente invención pueden producirse opcionalmente en células CHO o células bacterianas y, preferentemente, inhibir el crecimiento o la proliferación, o bien inducir la muerte de una célula a la que se unen. Con fines diagnóstico, los anticuerpos de la presente invención pueden marcarse de forma detectable, unirse a un soporte sólido, etc.

En otras formas de realización de la presente invención, la invención proporciona vectores que comprenden el ADN que codifica cualquiera de los anticuerpos anti-CD22 y anticuerpos anti-CD22 diseñados con cisteína que se detallan en la presente memoria descriptiva. También se proporcionan las células huésped que comprenden cualquiera de estos vectores. A modo de ejemplo, las células huésped pueden ser células CHO, células E. coli o células de levadura. También se proporciona un proceso para producir cualquier polipéptido detallado en la presente memoria descriptiva que comprende el cultivo de células huésped bajo las condiciones adecuadas para la expresión del polipéptido deseado y la recuperación de dicho polipéptido del cultivo celular.

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 WO2006034488. 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 WO2006/034488.

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. El antígeno asociado al tumor puede ser un factor de diferenciación de grupos (es decir, una proteína CD, incluyendo, entre otras, la CD22). Los anticuerpos anti-CD22 de la invención diseñados con cisteína conservan la capacidad de unión al antígeno de sus homólogos anticuerpos parentales anti-CD22. Por ende, los anticuerpos anti-CD22 de la invención diseñados con cisteína son capaces de unirse específica y preferentemente a los antígenos CD22, incluyendo las isoformas anti-CD22 humanas beta o alfa, o ambas; aún cuando dichos antígenos se expresan en la superficie celular, incluyendo, entre otras, las células B.

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.

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.

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:

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).

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 sin cisteína 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.

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.° 5342606; patente de EE.UU. N.° 5428155; patente de EE.UU. N.° 5316757; patente de EE.UU. N.° 5480990; patente de EE.UU. N.° 5462725; patente de EE.UU. N.° 5428139; patente de EE.UU. N.° 5385893; patente de EE.UU. N.° 5739294; patente de EE.UU. N.° 5750660; patente de EE.UU. N.° 5834456; 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 4737456), 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.

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.

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.

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)

El anticuerpo de la presente invención puede emplearse en cualquier método de ensayo conocido tales como ELISA, ensayos de unión competitiva, ensayos tipo "sandwich" directos e indirectos y ensayos de inmunoprecipitación (Zola, (1987) *Monoclonal Antibodies: A Manual of Techniques*, págs. 147-158, CRC Press, Inc.).

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.

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.

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).

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.

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.

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).

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.

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.

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:

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 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 Cys 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.

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:42-46:

CDKTHTGGGSQRLMEDICLPRWGCLWEDDF	Identificador de secuencia n.º:42
QRLMEDICLPRWGCLWEDDF	Identificador de secuencia n.º:43
QRLIEDICLPRWGCLWEDDF	Identificador de secuencia n.º:44
RLIEDICLPRWGCLWEDD	Identificador de secuencia n.º:45
DICLPRWGCLW	Identificador de secuencia n.º:46

Conjugados de anticuerpos con fármacos

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-CD22 mencionados anteriormente unidos covalentemente a un agente citotóxico o detectable.

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 y 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.

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.° 4970198; 5079233; 5585089; 5606040; 5693762; 5739116; 5767285; 5773001). Cantuzumab mertansina (Inmugen, 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.

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).

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

En algunas realizaciones, el inmunoconjugado comprende un anticuerpo de la invención (longitud completa o fragmentos) 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. 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.

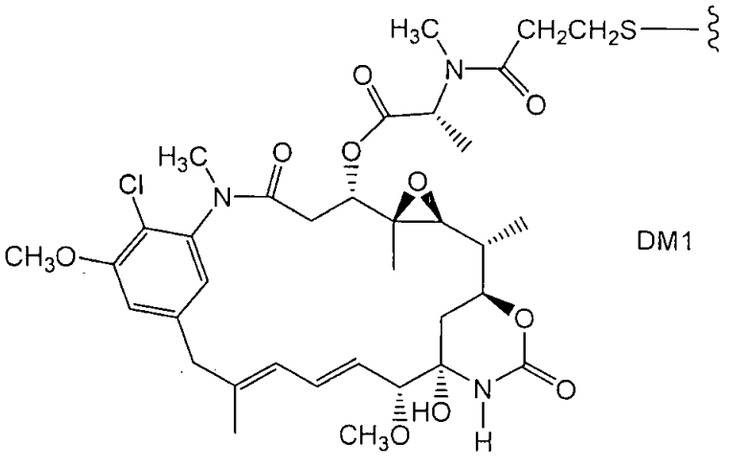
Los grupos de fármacos maitansinoides son grupos de fármacos que resultan atractivos para la elaboración de anticuerpos conjugados con fármacos por sus siguientes características: (i) son relativamente accesibles en lo que se refiere a su preparación mediante fermentación, modificación química o derivación 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.

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.

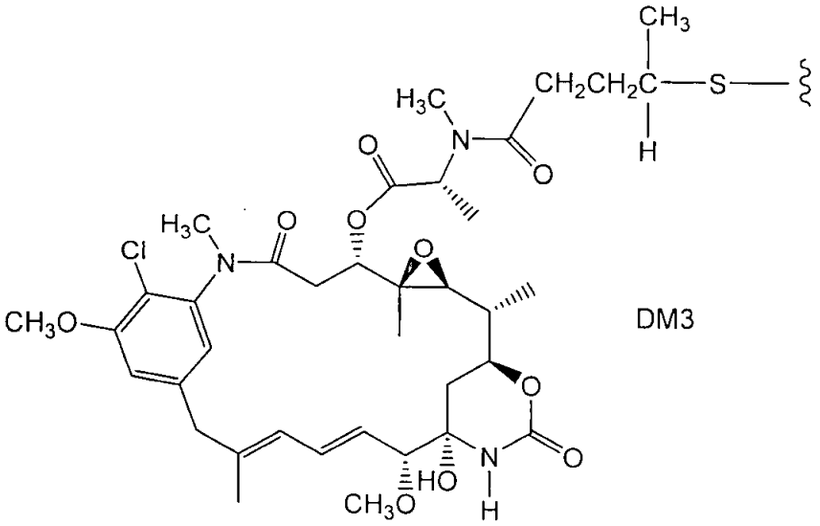
Entre los grupos ejemplares de fármacos maitansinoides están los que tienen un anillo aromático modificado, como por ejemplo: C-19-dicloro (patente de EE.UU. N.º 4256746) (preparado por reducción de ansamitocina P2 mediante hidruro de litio-aluminio); C-20-hidroxi (o C-20-dimetil) +/-C-19-dicloro (patentes 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.

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 (patentes de EE.UU. N.º 4371533) (preparado por la reducción de maitansinol mediante tricloruro de titanio/LAH).

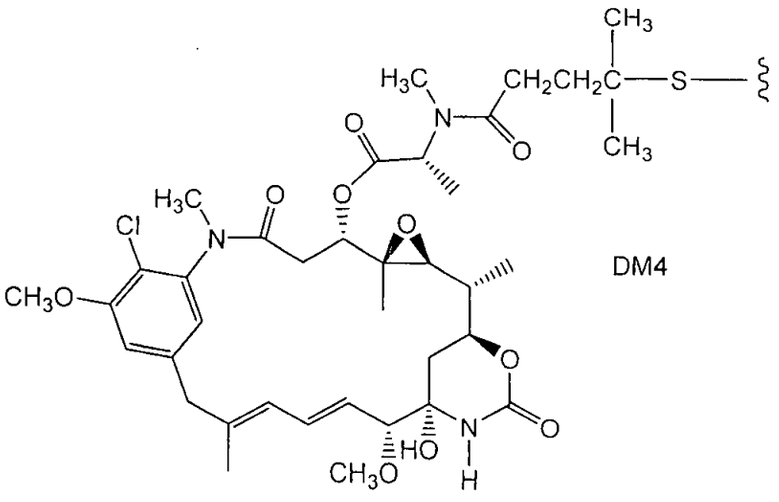
A continuación se citan algunos ejemplos de formas de realización ejemplares de grupos de fármacos maitansinoides: DM1; DM3; y DM4, con las estructuras:



DM1



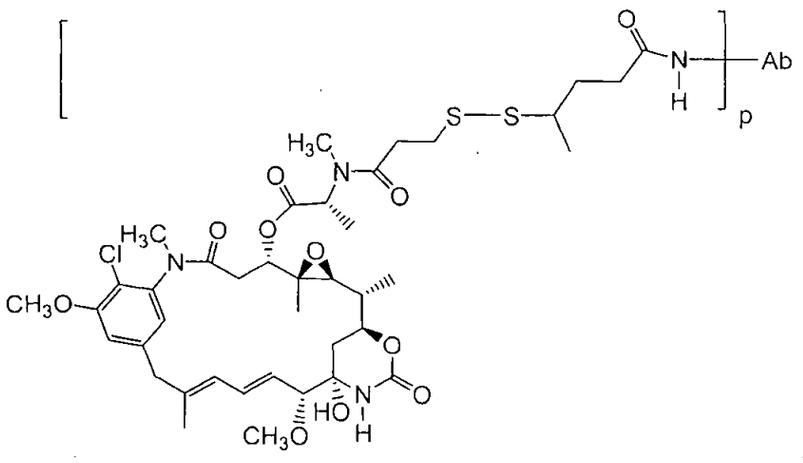
DM3



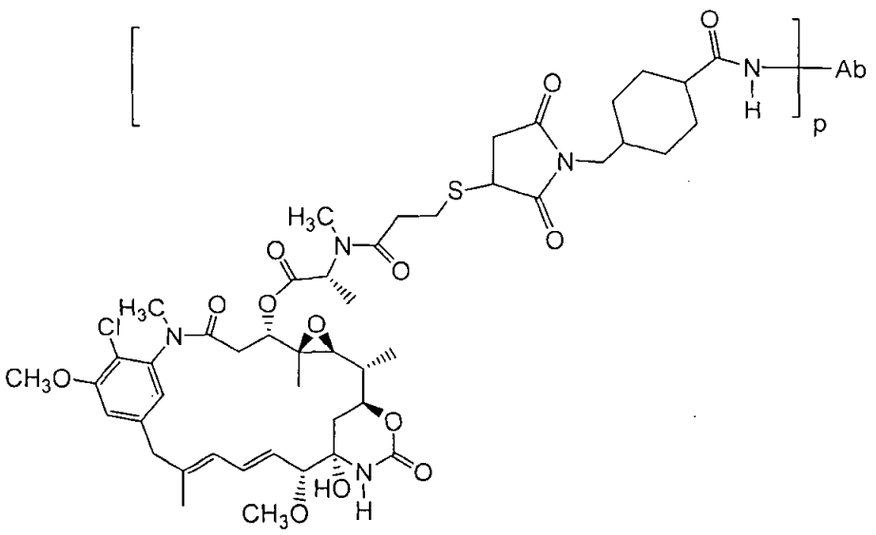
DM4

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, anticuerpo anti-HER2) 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.

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):

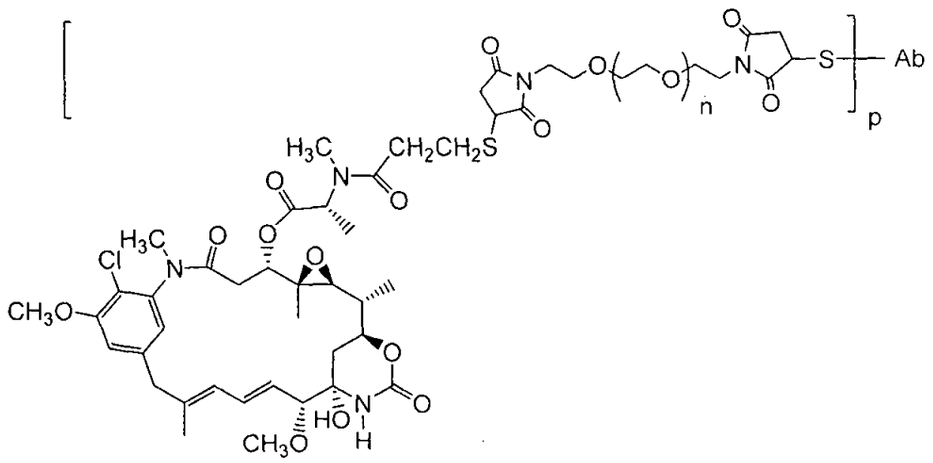


Ab -SPP-DM1



Ab-SMCC-DM1

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.

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, 6.441163 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.

Los conjugados de un anticuerpo con un maitansinoide anti-CD22 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.

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 5208020, 6441163 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.

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.

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 C-3 del maitansinol o un análogo del maitansinol.

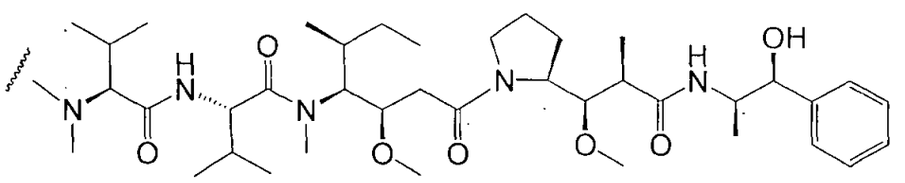
En una forma de realización, cualquiera de los anticuerpos de la invención (longitud completa o fragmentos) se conjuga con una o más moléculas de maitansinoides. En un forma de realización del inmunoconjugado, el agente citotóxico D es un maitansinoide DM 1. En una forma de realización del inmunoconjugado, el enlazador es SMCC. En una forma de realización, el conjugado fármaco-enlazador-anticuerpo es un anticuerpo anti-CD22 como se describe en la presente memoria descriptiva, al cuál está unido covalentemente a un agente citotóxico DM1 a través del enlazador SMCC.

Auristatinas y dolostatinas

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. ° 5635483; 5780588). 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.° 5663149) 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).

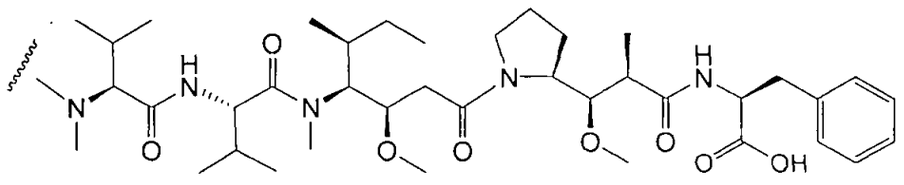
Entre las formas de realización ejemplares de auristatina están los grupos de fármacos DE y DF de monometilauristatina enlazados al extremo N-terminal, descritas en Senter y col., *Proceedings of the American Association for Cancer Research*, Volumen 45, Abstract Number 623, presentada el 28 de marzo de 2004, cuyo contenido se incorpora expresamente en su totalidad a modo de referencia.

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).



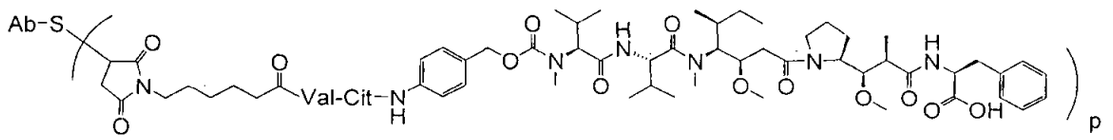
MMAE

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.° 2005/0238649):

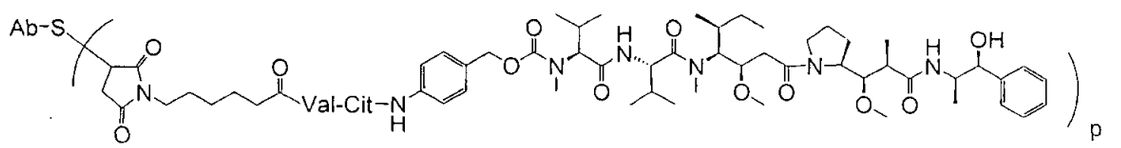


MMAF

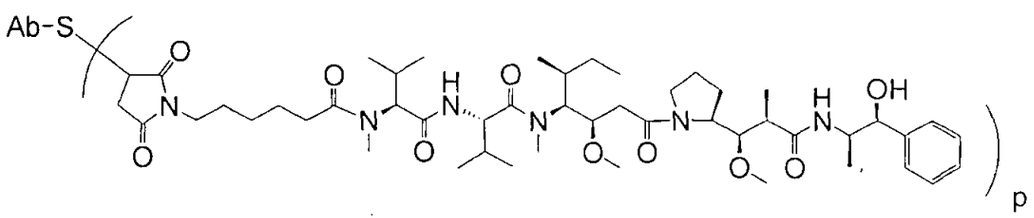
Otros ejemplos de realizaciones ejemplares que comprenden MMAE o MMAF y varios componentes enlazadores (descritos en la presente memoria en mayor detalle) tienen las siguientes estructuras y abreviaturas (donde Ab significa anticuerpo y p es 1 a aproximadamente 8):



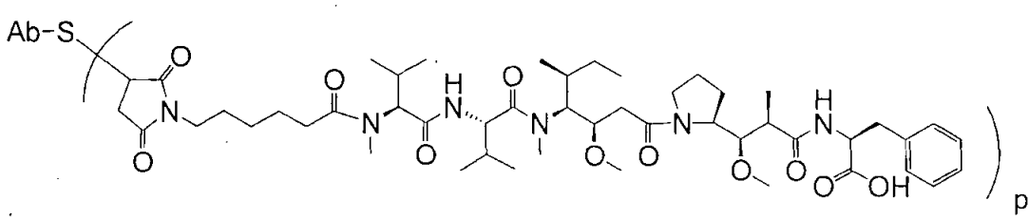
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

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 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. Biotechmol.* 21(7):778-784.

Caliqueamicina

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

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).

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.

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).

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}, Pb^{212} 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.

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.

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. ° 5.208.020).

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:

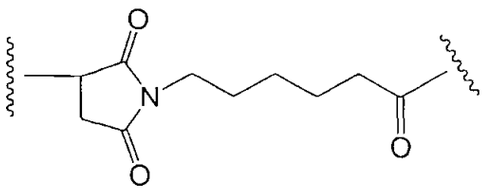
En los conjugados de anticuerpos con fármacos (ADC) de la invención, un anticuerpo (Ab) se conjuga con uno o más grúpos de fármacos (D), por ejemplo, grupos de fármacos de aproximadamente 1 a aproximadamente 20 por anticuerpo, a través de un enlazador (L). Los ADC de la formula I se pueden preparar por 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.

$$Ab-(L-D)_p \qquad \text{Fórmula I}$$

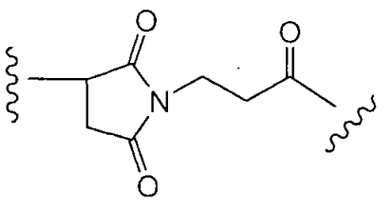
El enlazador puede estar formado por uno o más componentes enlazadores. Los componentes de enlace ejemplares incluyen: 6-maleimidocaproilo ("MC"), maleimidopropanoilo ("MP"), valina-citrulina ("val-cit"), alanina-fenilalanina ("ala-phe"), p-aminobensiloxicarbonil ("PAB"), N-Succinimidil 4-(2-piridiltio) pentanoato ("SPP"), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato ("SMCC"), y N-Succinimidil (4-yodo-acetil) aminobenzoato ("SIAB"). Los componentes enlazadores adicionales son conocidos en la materia y algunos se describen en la presente memoria descriptiva.

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 (gly-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.

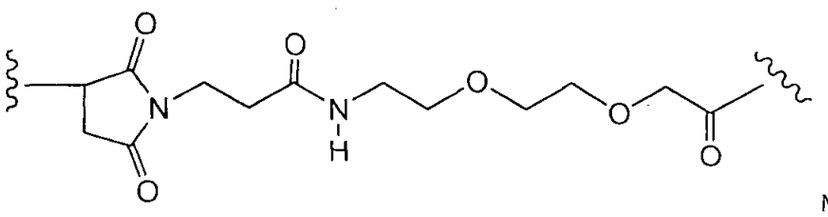
A continuación se muestran las estructuras ejemplares de los componentes enlazadores (donde la línea ondulada indica los sitios de enlace covalente a otros componentes del ADC)



MC

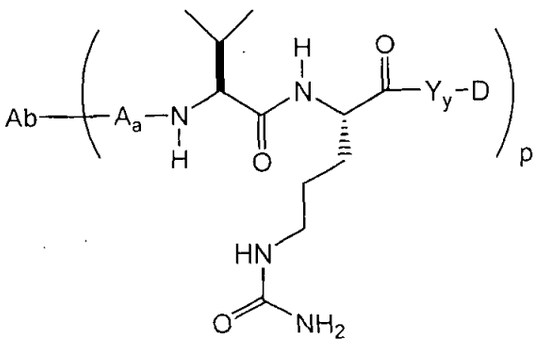


MP

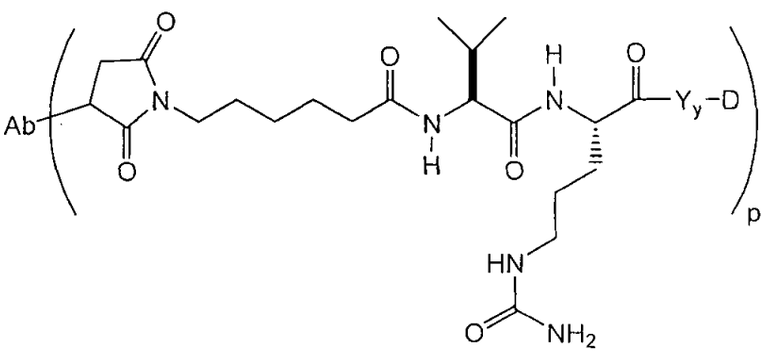


MPEG

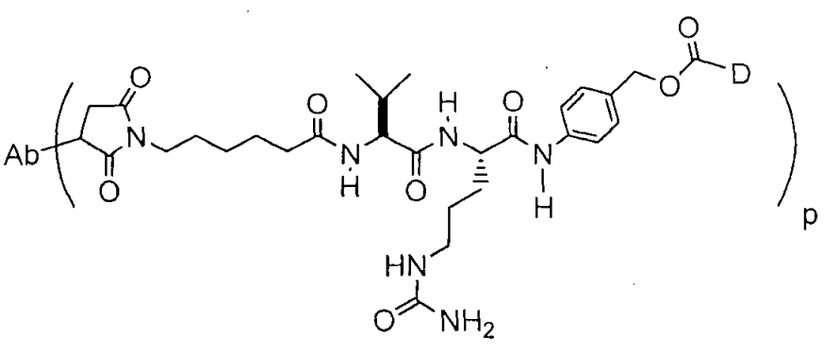
Los componentes enlazadores ejemplares adicionales y las abreviaciones incluyen (donde se muestra el anticuerpo (Ab) y el enlazador, y p es 1 a aproximadamente 8):



Val-cit



MC-val-cit



MC-val-cit-PAB

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 nucleofílicos 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).

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.° 5362852). Se puede hacer que este aldehído reaccione con una fracción de fármaco o un nucleófilo enlazador.

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.

En otro aspecto, el anticuerpo tiene uno o más residuos de lisina que pueden modificarse químicamente para introducir uno o más grupos sulfidrilos. La unidad del anticuerpo se enlaza a la unidad del enlazador mediante el átomo de sulfuro del grupo sulfidrilos. Entre los reactivos que pueden utilizarse

para modificar las lisinas se encuentran, entre otros, N-succimidil S-acetiltioacetato (SATA) y clorhidrato de 2-iminotiolano (reactivo de Traut).

En otra forma de realización, el anticuerpo puede tener uno o más grupos carbohidratos que pueden modificarse químicamente para obtener uno o más grupos sulfidrilos. La unidad del anticuerpo se enlaza a la unidad del enlazador, como la unidad de extensión, mediante el átomo de sulfuro del grupo sulfidrilos, tal como se describe en la presente memoria descriptiva.

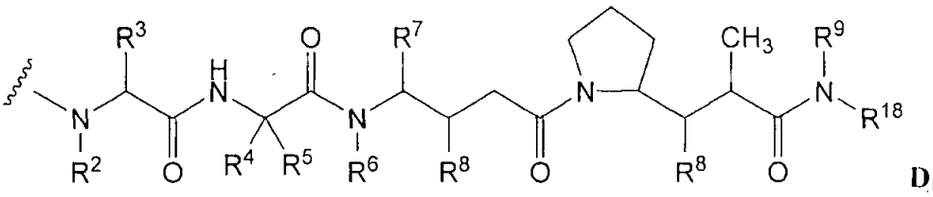
En otra forma de realización, el anticuerpo puede tener uno o más grupos carbohidratos que pueden oxidarse para proporcionar un grupo aldehído-(-CHO) (véase, por ejemplo, Laguzza, y col., *J. Med. Chem.* 1989, 32(3), 548-55). El aldehído correspondiente puede formar una unión con un punto reactivo en una unidad de extensión. Entre los puntos reactivos en una unidad de extensión que pueden reaccionar con un grupo carbonilo en un anticuerpo se incluyen, entre otros, hidracina e hidroxilamina. Otros protocolos para la modificación de proteínas para el enlace o la asociación con unidades de fármacos se describen en Coligan y col., *Current Protocols in Protein Science*, vol. 2, John Wiley y Sons (2002), incorporados en la presente memoria descriptiva a modo de referencia.

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.° 5.208.020; patente de EE.UU. N.° 6.441.163; documentos WO2005037992, WO2005081711 y WO2006/034488, los cuales se incorporan expresamente en su totalidad a modo de referencia.

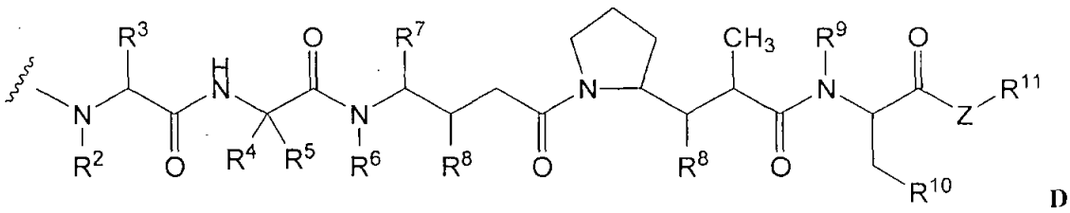
Alternativamente, es posible realizar una fusión de proteínas que contengan el anticuerpo y el agente citotóxico, por ejemplo mediante técnicas recombinantes o síntesis de péptidos. La longitud del ADN puede incluir regiones respectivas que codifiquen las dos porciones del conjugado, ya sea adyacentes entre sí o separados por una región que codifica un péptido de enlace, el cual no destruye las propiedades deseadas del conjugado.

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).

En una forma de realización del inmunoconjugado, el agente citotóxico, D, es una auristatina de fórmula D_E o D_F



D_E



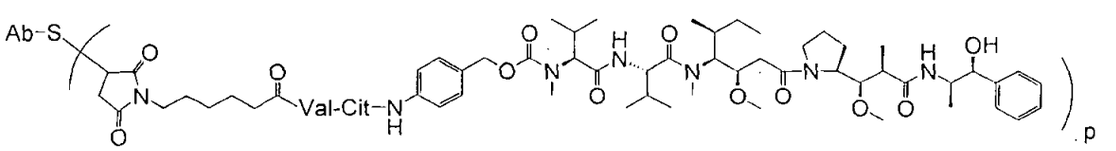
D_F

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

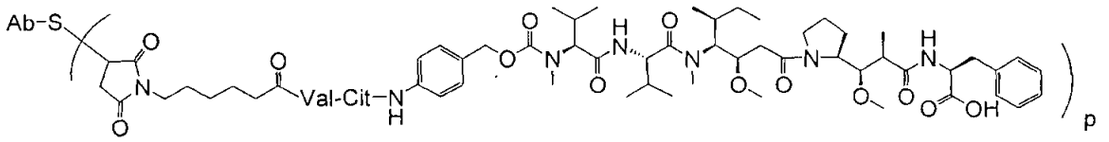
(d) p oscila entre aproximadamente 1 y 8.

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.

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-CD22 anteriores, S es un átomo de sulfuro y p oscila entre 2 y 5. En una forma de realización, el inmunoconjugado tiene la fórmula



donde Ab es cualquiera de los anticuerpos anti-CD22 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:

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:

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:

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 B, en el cual dicho método comprende la administración a un sujeto de la composición farmacéutica. En una forma de realización, el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. En una forma de realización, el trastorno de proliferación celular se asocia a un aumento de la expresión de CD22 en la superficie de una célula.

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 CD22. En una forma de realización, la célula B es una célula tumoral. En una forma de realización, la célula tumoral es una célula B de un mamífero que experimenta o se cree que experimenta el trastorno proliferativo de células B seleccionada a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. La célula 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*.

En un aspecto, se proporciona un método de utilización del anticuerpo anti-CD22 para efectuar un ensayo de suero soluble CD22 en un mamífero que experimenta leucemia o un linfosoma para diagnosticar leucemia de las células B o linfoma de las células B, midiendo la progresión clínica o la regresión de las enfermedades, o evaluando la carga o recurrencia del tumor. Estos métodos se describen en la patente de EE.UU. N.º 20050244828 (Kreitman, R.J. y col., la totalidad de los contenidos de éste se incorporan en la

presente memoria descriptiva a modo de referencia) utilizando un conjugado de anticuerpo PE38 anti-CD22 RFB4 (fragmento 38 de exotoxina A de Pseudomonas) con toxina (véase R.J. y col., NEJM 345:241-247 (2001)).

BREVE DESCRIPCIÓN DE LAS FIGURAS

Figuras de 1A a 1D: La **Figura 1A** es un diagrama de CD22 que indica los siete dominios de tipo inmunoglobulina del dominio extracelular de la isoforma beta. La isoforma alfa carece de los dominios 3 y 4. "TM" se refiere al dominio transmembrana. La **Figura 1B** describe la secuencia de aminoácidos de la forma beta de CD22 (identificador de secuencia n.°: 27). La forma alfa de CD22 carece de los aminoácidos que se muestran en letra cursiva (que codifican los dominios 3 y 4 del dominio extracelular). El dominio extracelular de la forma madura de la proteína se muestra subrayado (identificador de secuencia n.°: 28). Los aminoácidos del 1 al 21 describen la secuencia de señal escindida de la forma madura. La **Figura 1C** constituye la secuencia de aminoácidos de CD22 alfa (identificador de secuencia n.°: 29). El dominio extracelular (ECD) de CD22 alfa se muestra subrayado (identificador de secuencia n.°: 30). La **Figura 1D** constituye la secuencia de aminoácidos de CD22 del mono cinomolgus (identificador de secuencia n.° 31). Los primeros 19 aminoácidos de CD22 de mono cinomolgus es la secuencia de señal.

Figuras 2A y 2B: La **Figura 2A** describe la secuencia de aminoácidos de la región variable de la cadena pesada del anticuerpo anti-CD22 10F4 murino de la invención (m10F4) que se alinea con el anticuerpo 1 de la versión 10F4 humanizado (h10F4v1) y con la secuencia del subgrupo humano III. Las regiones hipervariables (HVR) se muestran en un recuadro (HVR-H1, HVR-H2, HVR-H3). Las secuencias que agrupan las HVR corresponden a las secuencias de estructuras (de FR-H1 a FR-H4). Las secuencias se enumeran de acuerdo con la numeración de Kabat. Las CDR de Kabat, Chothia y de contacto se indican en las HVR recuadradas. La **Figura 2B** describe la secuencia de aminoácidos de la región variable de la cadena ligera del anticuerpo anti-CD22 10F4 murino de la invención (m10F4) que se alinea con el anticuerpo 1 de la versión 10F4 humanizado (h10F4v1) y con la secuencia kappa humana I. Las versiones 2 y 3 del anticuerpo 10F4 humanizado (h10F4v2 y h10F4v3) tienen la misma secuencia de aminoácidos para la forma madura secretada. Los anticuerpos h10F4v2 y h10F4v3 difieren del h10F4v1 en el aminoácido 28 de la HVR-L1 (N28V). Las HVR se muestran en recuadros. Las secuencias FR-L1, FR-L2, FR-L3 y FR-L4 agrupan las HVR (HVR-L1, HVR-L2, HVR-L3). Las secuencias se enumeran de acuerdo con la numeración de Kabat. Las CDR de Kabat, Chothia y de contacto se indican en las HVR recuadradas.

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, 47, 48, 7).

- estructuras "B", "C" y "D" de consenso del subgrupo I humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 50, 51, 52, 7; identificadores de secuencia n.º: 50, 51, 52, 7; e identificadores de secuencia n.º: 50, 51, 53, 7).

- estructura "A" de consenso del subgrupo II humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 54, 55, 56, 7).

- estructuras "B", "C" y "D" de consenso del subgrupo II humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 57, 58, 56, 7; identificadores de secuencia n.º: 57, 58, 59, 7; e identificadores de secuencia n.º: 57, 58, 60, 7).

- estructura "A" de consenso del subgrupo III humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 61, 62, 63, 7).

- estructuras "B", "C" y "D" de consenso del subgrupo III humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 64, 65, 63, 7; identificadores de secuencia n.º: 64, 65, 66, 7; e identificadores de secuencia n.º: 64, 65, 67, 7).

- estructura "A" del aceptor 1 humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 68, 62, 69, 7).

- estructuras "B" y "C" del aceptor humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 64, 65, 69, 7; e identificadores de secuencia n.º: 64, 65, 70, 7).

- estructura "A" del aceptor 2 humano de VH menos las CDR de Kabat (identificadores de secuencia n.º: 68, 62, 71, 7).

- estructuras "B", "C" y "D" del aceptor 2 humano de VH menos las regiones hipervariables extendidas (identificadores de secuencia n.º: 64, 65, 71, 7; identificadores de secuencia n.º: 64, 65, 72, 7; e identificadores de secuencia n.º: 64, 65, 73, 7).

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: 74, 75, 76, 77

- estructura de consenso del subgrupo I kappa humano de VL (κv1): identificadores de secuencia n.º: 74, 78, 76, 77

- estructura de consenso del subgrupo II kappa humano de VL (κv2): identificadores de secuencia n.º: 49, 79, 80, 77

- estructura de consenso del subgrupo III kappa humano de VL (κv3): identificadores de secuencia n.º: 81, 82, 83, 77

- estructura de consenso del subgrupo IV kappa humano de VL (κv4): identificadores de secuencia n.º: 84, 85, 86, 77

Figuras 5A y 5B: La **Figura 5A** 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: 38; y alotipos A, donde la secuencia de aminoácidos SREEM en el identificador de secuencia n.º: 38 cambia a SRDEL), humIgG2 (identificador de secuencia n.º 39), humIgG3 (identificador de secuencia n.º: 40) y humIgG4 (identificador de secuencia n.º: 41) 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. La **Figura 5B** representa las secuencias de aminoácidos de longitud completa (regiones variables y constantes) de las cadenas ligera y pesada del anticuerpo anti-CD22 10F4v2 humanizado, isotipo IgG1. Las partes subrayadas representan los dominios constantes.

Las **Figuras de la 6A a la 6D** muestran los resultados de los ensayos que miden diversos determinantes de la eficacia del anticuerpo CD22 conjugado con fármacos en las líneas celulares de linfomas. La **Figura 6A** indica que los niveles más elevados de la superficie celular CD22 se correlacionan con un nivel más bajo del anticuerpo anti-CD22-MCC-DM1 IC50 (mayor eficacia). La **Figura 6B** indica que la mayor internalización del anti-CD22-MCC-DM1 se correlaciona con un anti-CD22-MCC-DM1 IC50 menor. La **Figura 6C** indica que una mayor sensibilidad intrínseca de las células sin fármacos se correlaciona con un anti-CD22-MCC-DM1 IC50 menor. La **Figura 6D** es un

fotomicrográfo que muestra la internalización del anticuerpo anti-CD22 marcado fluorescentemente después de la unión a CD22 en la superficie celular.

Figuras 7A y 7B: La **Figura 7A** es un gráfico de la reducción de volumen del tumor *in vivo* en un modelo xenotransplantado que muestra que la administración de un anticuerpo anti-CD22 mu10F4-smcc-DM1 y hu10F4v1-smcc-DM1 a un ratón SCID con tumores de células B humanas redujeron considerablemente el volumen tumoral. La concentración del fármaco fue aproximadamente 4 y 4,6, véase la Tabla 4. La **Figura 7B** es un gráfico de un estudio similar, pero la concentración del fármaco fue ligeramente inferior a aproximadamente 2,9 y 3,0 (véase la Tablea 5), y la eficacia de mu10F4-smcc-DM1 y hu10F4v2-smcc-DM1 se comparó con el anticuerpo de control y el mu10F4 no conjugado. La **Figura 7C** es un gráfico sobre la reducción del tumor *in vivo* en un modelo xenotransplantado en el que el anti-CD22-spp-DM1 se administró según se indica en la Tabla 6.

Figuras 8A y 8B: La **Figura 8A** es un gráfico de anticuerpos anti-CD22 5E8.1.8-smcc-DM1 y RFB4-smcc-DM1 que se administraron a xenotransplantes de células Ramos. La **Figura 8B** es un gráfico de anticuerpos anti-CD22 5E8.1.8-smcc-DM1 y RFB4-smcc-DM1 que se administraron a xenotransplantes de células BJAB-luc.

La **Figura 9** es un gráfico que muestra el efecto relativo en el volumen tumoral con el transcurso del tiempo después de la administración del anti-CD22 (RFB4)-smcc-DM1 en concentraciones bajas, medias y altas de fármacos.

La **Figura 10** es un gráfico que muestra el efecto relativo en el volumen tumoral con el transcurso del tiempo después de la administración del anti-CD22 (RFB4)-MC-vcPAB-MMAF o anti-CD22 (RFB4)-MC-MMAF en xenotransplantes Ramos.

La **Figura 11** es un gráfico que muestra el efecto relativo en el volumen tumoral con el transcurso del tiempo después de la administración del anti-CD22(RFB4)-smcc-DM1 o -MCvcPAB-MMAE.

La **Figura 12** es un gráfico que muestra el efecto relativo en el volumen tumoral con el transcurso del tiempo después de la administración de las variantes anti-CD22 10F4 humanizadas tales como los inmunoconjugados MMAF o DM1, tal como se describen en la Tabla 12.

Las **Figuras de la 13A a la 13C** son gráficos que muestran el efecto relativo en el volumen tumoral en el tiempo después de la administración del anti-CD22-smcc-DM1 o anti-CD22-MC-MMAF en diferentes modelos de xenotransplantes de linfomas de células B: SuDHL-4 (Figura 13A), DoHH2 (Figura 13B) y Granta-519 (Figura 13C).

La **Figura 14** muestra diagramas de los dominios CD22 eliminados del mapeo de epítopos, tal como se describe en los ejemplos. Los dominios se enumeran del 1 al 7. "TM" se refiere al dominio transmembrana.

La **Figura 15** muestra las representaciones de los conjugados de anticuerpo anti-CD22 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).

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-CD22 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-CD22 diseñado con cisteína con fármacos (ADC).

Las **Figuras de la 17A a la 17C** representan las secuencias de aminoácidos de anticuerpos anti-CD22 de la invención diseñados con cisteína, en los que la cadena ligera, la cadena pesada o la región Fc se modifican para diseñar una cisteína en las posiciones determinadas de aminoácidos. La **Figura 17A** representa un secuencia de aminoácidos de la región variable de la cadena ligera anti-CD22 10F4 en la que una valina en la posición 205 de la numeración de Kabat (posición secuencial 210 de la valina) se modifica a una cisteína. La **Figura 17B** representa un secuencia de aminoácidos de la región variable de la cadena pesada anti-CD22 10F4 en la que una alanina en la posición 118 de la numeración EU (posición secuencial 121 de la alanina) se modifica a una cisteína. La **Figura 17C** representa un secuencia de aminoácidos de la región variable de Fc anti-CD22 10F4 en la que una serina en la posición 400 de la enumeración EU (posición secuencial 403 de la serina) se modifica a una cisteína. En cada figura, el aminoácido modificado se muestra en un texto en negrita con doble subrayado. Los subrayados simples indican regiones constantes. Las regiones variables no están subrayadas.

Las **Figuras de la 18A a la 18E** son diagramas FACS que indican que la unión de los conjugados de thiomab anti-CD22 con fármacos (TDC) de la invención se unen a CD22 expresado en la superficie de células BJAB-lucs es similar a las regiones variables de LC, HC y Fc de thiomab, así como a los diferentes conjugados de fármacos mostrados.

La **Figura 19** es un gráfico que indica los cambios en el volumen medio del tumor que se produjeron en el tiempo en un modelo xenotransplantado tratado con diversos conjugados de thiomab anti-CD22 con fármacos, que variaron según la posición de la cisteína diseñada (LC, HC o Fc) o según el

conjugado de fármaco (MMAF o MMAE), o ambos. Los modelos xenotransplantados tratados con conjugados de thiomab anti-CD22 con fármacos 10F4-LC-V210C-MCvcPAB-MMAE y anti-CD22 10F4-HC-A121C-MCvcPAB-MMAE indicaron una reducción en el volumen tumoral durante el estudio.

La **Figura 20A** es un gráfico que indica cambios en el volumen medio del tumor producidos durante el transcurso del tiempo en un xenotransplante de linfoma de células del manto Granta-519 humanas en ratones CB17 SCID tratados con conjugados de thiomab anti-CD22 A118C de cadena pesada con fármacos conjugados a diversos grupos de fármacos del enlazador o administrados en diferentes dosis, o ambos, tal como se muestra. Los conjugados de thiomab anti-CD22 10F4-HC (A118C)-MCvcPAB-MMAE con fármacos aparentemente son los agentes de prueba más eficaces de este experimento. La **Figura 20B** es un gráfico que indica los cambios en el volumen medio del tumor producidos en el tiempo en un xenotransplante DOHH2 de linfoma folicular en ratones CB17 SCID tratados con los mismos conjugados de thiomab anti-CD22 con fármacos A118C de cadena pesada, pero en dosis más altas. Los conjugados de thiomab anti-CD22 10F4-HC (A118C)-MCvcPAB-MMAE con fármacos aparentemente son los agentes de prueba más eficaces de este experimento. La **Figura 20C** es un diagrama sobre el porcentaje de cambios de peso en los ratones utilizados en el estudio del xenotransplante DOHH2 que muestra que no se produjo ningún cambio de peso significativo durante los primeros 14 días del estudio.

Las **Figuras 21A y 21B** son gráficos de barras que muestran los cambios de AST (aspartato aminotransferasa) en el suero (**Figura 21A**) y neutrófilos en el suero (**Figura 21B**) en los días 0 y 5, durante los que se administraron los conjugados de anticuerpos con fármacos que contienen enlazadores escindibles y no escindibles.

Las **Figuras 22A y 22B** son gráficos que muestran la reducción de células B periféricas (células $CD20^+$) en monos cinomolgus a los que se les suministró una dosis de 10, 20 y 30 mg/kg de anti-CD22 MMAF (**Figura 22A**) y anti-CD22 DM1 (**Figura 22B**).

Las **Figuras 23A y 23B** son gráficos que no muestran ningún cambio significativo en los linfocitos $CD4^+$ con dosis de 10, 20 y 30 mg/kg de anti- CD22 MMAF (**Figura 23A**) y anti-CD22 DM1 (**Figura 23B**).

Las **Figuras 24A y 24B** indican muestras histológicas del tejido tonsilar del mono cinomolgus, en el que la reducción de las células B del centro germinal, que se manifiestan en el control vehicular (**Figura 24A**), se reducen en una muestra tonsilar de un animal al que se le suministró una dosis de 10 mg/kg de hu10F4v3-SMCC-DM1.

La **Figura 25A** es un diagrama que indica las regiones de folículos esplénicos de donde se obtuvieron las muestran para un estudio en el que se demostró que los conjugados de anticuerpos anti-CD22 con fármacos conservan las células B en el tejido en reposo en los monos cinomolgus. Las células en división en el centro germinal de los folículos esplénicos de mono cinomolgus se redujeron a células germinales en división de los bazos de estos animales, a los que se les administró una dosis de 10 mg/kg de hu10F4v3-MC-MMAF (**Figuras 25B y 25C**). Las células B vírgenes no en división no se redujeron bajo las mismas condiciones (**Figuras 25D y 25E**).

DESCRIPCIÓN DETALLADA DE LAS FORMAS DE REALIZACIÓN DE LA INVENCIÓN

Se proporcionan anticuerpos aislados que se unen a CD22. Se proporcionan, además, inmunoconjugados que comprenden anticuerpos anti-CD22. También se proporcionan anticuerpos anti-CD22 diseñados con cisteína y sus inmunoconjugados. 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 CD22. 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, cómo un tumor o cáncer. En determinadas formas de realización, los anticuerpos o inmunoconjugados de la invención son útiles para la detección de CD22, como por ejemplo, CD22 expresado en la superficie celular.

Se proporcionan polinucleótidos que codifican anticuerpos anti-CD22. Se proporcionan vectores que comprenden polinucleótidos que codifican anticuerpos anti-CD22, así como células huésped que comprenden dichos vectores. También se proporcionan composiciones, incluyendo las formulaciones farmacéuticas, que comprenden uno o más de los polinucleótidos, anticuerpos anti-CD22 o inmunoconjugados de la invención.

Técnicas generales

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 y 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

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.

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.

Por "purificada" se entiende una molécula que está presente en una muestra a una concentración de al menos el 98% de su peso, o al menos el 98% del peso de la muestra en la que está contenida.

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.

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.

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 huésped 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 huésped al introducirse en ésta y, de este modo, se replican al mismo tiempo que el genoma del huésped. 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.

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.

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.

"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.° de Registro de Derechos de Autor de Estados Unidos TXU510087. El programa ALIGN-2 está comercializado por Genetech, 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.

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.

En la presente memoria descriptiva, un "marcador de la superficie de células B" o "antígeno de la superficie de células B" es un antígeno que se expresa en la superficie de una célula B que puede actuar con un antagonista que se une a él, incluyendo, entre otros, anticuerpos a un antígeno de la superficie de las células B o un antígeno de la superficie de las células B en forma soluble capaz de unir antagonistas de un ligando a un antígeno de las células B que se produzca naturalmente. Entre los marcadores ejemplares de la superficie de células B se encuentran los marcadores de la superficie de leucocitos CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 y CD86 (para conocer sus descripciones, consulte *The Leukocyte Antigen Facts Book*, 2ª edición, 1997, ed. Barclay y col. Academic Press, Harcourt Brace & Co., New York). Otros marcadores de la superficie de células B incluyen el RP105, el FcRH2, el CR2 de células B, el CCR6, el P2X5, el HLA-DOB, el CXCR5, el FCER2, el BR3, el BAFF, el BLyS, el Btig, el NAG14, el SLGC16270, el FcRH1, el IRTA2, el ATWD578, el FcRH3, el IRTA1, el FcRH6, el BCMA y el 239287. El marcador de la superficie de células B de interés particular se expresa preferentemente en las células B en comparación con los otros tejidos del mamífero que no pertenecen a células B, y puede expresarse tanto en células B precursoras como maduras.

En la presente memoria descriptiva, "CD22" se refiere a cualquier CD22 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 CD22 no procesado de "longitud completa", así como cualquier forma de CD22 que derive del procesamiento en

la célula. El término también engloba variantes de CD22 presentes naturalmente, por ejemplo, variantes de ayuste, variantes alélicas o isoformas. La isoforma principal del CD22 (CD22 beta) comprende los aminoácidos 847 y siete regiones de tipo inmunoglobulina en el dominio extracelular (véase Wilson, G.L. y col., *J. Exp. Med.* 173:137-146 (1991)). Una isoforma secundaria, CD22 alfa, comprende los aminoácidos 647 y carece de los dominios 3 y 4 de tipo inmunoglobulina en el dominio extracelular (véase Stamenkovic, I. y Seed, B., *Nature* 345:74-77 (1990)) y Wilson y col., (1991), mencionado anteriormente). La secuencia de aminoácidos del CD22 beta se describe en la Figura 1B, en la que la parte subrayada representa el dominio extracelular (ECD) y la parte en letra cursiva indica los aminoácidos ausentes en la secuencia del dominio extracelular del CD22 alfa. La Figura 1C muestra la secuencia de aminoácidos del CD22 alfa en el que se subraya el dominio extracelular. La secuencia de aminoácidos del aminoácido 1 al 21 representa la secuencia de señal escindida de la forma madura de la proteína. En una forma de realización, el CD22 se expresa en la superficie celular, como por ejemplo, en la superficie de una célula B normal o una célula B tumoral. La Figura 1D muestra la secuencia de aminoácidos del CD22 del mono cinomolgus.

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.

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.

Por "anticuerpo anti-CD22" o "anticuerpo que se une a CD22" se entenderá un anticuerpo con capacidad de unión a CD22, con suficiente afinidad para que dicho anticuerpo sea útil como agente diagnóstico o terapéutico, o ambos, para actuar contra CD22. Preferiblemente, el alcance de la unión de un anticuerpo anti-CD22 a una proteína no afín no relacionada con CD22 presenta menos de un 10% aproximadamente de unión del anticuerpo a CD22, según medición, por ejemplo, mediante radioinmunoensayo (RIA). En ciertas formas de realización, un anticuerpo que se une a CD22 tiene una

constante de disociación (Kd) de ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM o ≤ 0,1 nM. En ciertas formas de realización, un anticuerpo anti-CD22 se une a un epítopo de CD22 que se conserva entre los CD22 de distintas especies.

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.

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.

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 función de las secuencias de aminoácidos de sus dominios constantes.

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.

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.

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.

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.

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.

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.

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).

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.

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.

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).

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.º 4.816.567 y Morrison y col., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

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).

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.

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.

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

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 (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 y HVR-H2 de los anticuerpos anti-CD22 10F4 de la invención son H26-H35 y H49-H65 según la numeración de Kabat.

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.

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.

Un "aminoácido sin cisteína" se refiere a un residuo de aminoácido sin cisteína que se ha diseñado en un anticuerpo parental y tiene un grupo tiol funcional (-SH), y no se empareja con un enlace disulfuro untramolecular o intermolecular, ni forma parte de éste.

El término "valor de reactividad del tiol" es una caracterización cuantitativa de la reactividad de aminoácidos sin cisteína. El valor de reactividad del tiol es el porcentaje de aminoácidos sin cisteína en un anticuerpo diseñado con cisteína que reacciona con un reactivo de tiol y se convierte en un valor máximo

de 1. Por ejemplo, un aminoácido sin cisteína en un anticuerpo diseñado con cisteína que reacciona en el 100% y se produce con un reactivo de tiol, como por ejemplo un reactivo de biotina-maleimido, para formar un anticuerpo marcado con biotina tiene un valor de reactividad del tiol de 1,0. Otro aminoácido diseñado con cisteína en el mismo o en otro anticuerpo parental que reacciona en un 80% y se produce con un reactivo de tiol tiene un valor de reactividad del tiol de 0,8. Otro aminoácido diseñado con cisteína en el mismo o en otro anticuerpo parental que no reacciona con un reactivo de tiol tiene un valor de reactividad del tiol de 0. La determinación del valor de reactividad del tiol de una cisteína determinada puede realizarse mediante el ensayo ELISA, la espectroscopía de masas, la cromatografía líquida, la autorradiografía u otras pruebas analíticas cuantitativas. Los reactivos de tiol que permiten capturar el anticuerpo diseñado con cisteína y comparar y cuantificar la reactividad de la cisteína incluyen biotina-PEO-maleimido ((+)-biotinil-3-maleimidopropionamidil-3,6-dioxaoctainediamino, Oda y col., (2001) *Nature Biotechnology* 19:379-382, Pierce Biotechnology, Inc.) Biotina-BMCC, PEO-Iodoacetil Biotina, Iodoacetil-LC-Biotina y Biotina-HPDP (Pierce Biotechnology, Inc.) y Nα-(3-maleimidilpropionilo)biocitina (MPB, Molecular Probes, Eugene, OR). Otras fuentes comerciales para los reactivos enlazadores de biotinilación, bifuncionales y multifuncionales incluyen Molecular Probes, Eugene, OR, y Sigma, St. Louis, MO

Un "anticuerpo parental" es un anticuerpo que comprende una secuencia de aminoácidos en la que se sustituyen uno o más residuos de aminoácidos por uno o más residuos de cisteínas. El anticuerpo parental puede comprender una secuencia de tipo natural o nativa. El anticuerpo parental puede contener cambios preexistentes en las secuencias de aminoácidos (como por ejemplo, adiciones, eliminaciones o sustituciones, o todas las anteriores) con respecto a otra secuencia de tipo natural o nativa, o formas modificadas de un anticuerpo. Un anticuerpo parental puede dirigirse contra un antígeno diana de interés, como por ejemplo, un polipéptido biológicamente importante. También se contemplan los anticuerpos dirigidos contra antígenos no polipéptidos (como los antígenos glicolípidos asociados al tumor; véase patente de EE.UU. N.° 5091178).

Las abreviaturas que se utilizan en la presente memoria descriptiva se definen de la siguiente manera: BME es beta-mercaptoetanol, Boc es N-(t-butoxicarbonil), cit es citrulina (ácido 2-amino-5-ureido pentanóico), dap es 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(ácido 2-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, PAB es p-aminobenzilcarbamoil, PBS es solución salina fosfatada (pH 7), 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.

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).

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.

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.

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.

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.

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 FcRn 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.

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).

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.

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).

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).

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).

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.

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.

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.

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: 1)-HVR-H1-WVRQAPGKGLEWV (FR-H2, identificador de secuencia n.º: 3)-HVR-H2-RFTISADTSKNTAYLQMNSLRAEDTAVYYC (FR-H3, identificador de secuencia n.º: 5)-HVR-H3-WGQGTLVTVSS (FR-H4, identificador de secuencia n.º: 7).

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: 8)-HVR-L1-WYQQKPGKAPKLLIY (FR-L2, identificador de secuencia n.º: 11)-HVR-L2-GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (FR-L3, identificador de secuencia n.º: 13)-HVR-L3-FGQGTKVEIK (FR-L4, identificador de secuencia n.º: 15).

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 proteína 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 huésped 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 huésped, 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.

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.

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.

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.

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).

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 los trastornos proliferativos de células B o los tumores de las células B, o ambos, por ejemplo, linfoma, linfoma no Hogkins (LNH), LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

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.

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.

Los términos "cáncer" y "cancerígeno" describen o se refieren al estado fisiológico de los mamíferos típicamente caracterizado por un crecimiento/proliferación celular no regulado. Los ejemplos de cáncer incluyen, entre otros, trastornos proliferativos de células B que se seleccionan a partir del linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. Otros ejemplos de cáncer son carcinoma, linfoma (por ejemplo, linfoma de Hodgkin y linfoma no Hodgkin), blastoma, sarcoma y leucemia. Ejemplos más concretos de estas clases de

cáncer son el cáncer de células escamosas, el cáncer de células pulmonares pequeñas, el adenocarcinoma pulmonar, el carcinoma pulmonar escamoso, el cáncer de peritoneo, el cáncer hepatocelular, el cáncer gastrointestinal, el cáncer pancreático, el glioma, el cáncer de cuello uterino, el cáncer de ovario, el cáncer de hígado, el cáncer de vejiga, el hepatoma, el cáncer de mama, el cáncer de colon, el cáncer colorrectal, el carcinoma endometrial o uterino, el carcinoma de las glándulas salivales, el cáncer de riñón, el cáncer de hígado, el cáncer de próstata, el cáncer vulval, el cáncer de tiroides, el carcinoma hepático, la leucemia y otros trastornos linfoproliferativos y diversos tipos de cáncer de cabeza y cuello.

En la presente memoria descriptiva, una "neoplasia maligna de las células B" incluye el linfoma no Hodgkin (LNH), incluyendo LNH folicular de bajo grado, LNH linfocítico de célula pequeña (SL), LNH folicular de grado intermedio, LNH difuso de grado intermedio, LNH inmunoblástico de grado alto, LNH linfoblástico de grado alto, LNH de células pequeñas no divididas de alto grado, LNH voluminoso, linfoma de células del manto, linfoma relacionado con el SIDA, macroglobulinemia de Waldenstrom, linfona no Hodgkin (LNH), enfermedad de Hodgkin con predominio linfocítico (EHPL), linfoma linfocítico de células pequeñas (LLCP), leucemia linfocítica crónica (LLC), LNH indolente, incluyendo LNH indolente recurrente y LNH indolente resistente a rituximab, leucemia, incluyendo leucemia linfoblástica aguda (LLA), leucemia linfocítica crónica (LLC), leucemia de células pielosas, leucemia mieloblástica crónica, linfoma de células del manto y otros tumores hematológicos. Dichos tumores pueden tratarse con anticuerpos dirigidos contra los marcadores de la superficie de células B, como los CD22. Estas enfermedades que se consideran en la presente memoria descriptiva se tratan mediante la administración de un anticuerpo dirigido contra un marcador de la superficie de células B, tal como el CD22 e incluye la administración de un anticuerpo no conjugado ("desnudo") o un anticuerpo conjugado con un agente citotóxico, tal como se describe en la presente memoria descriptiva. Las enfermedades que se consideran en la presente memoria descriptiva también se tratan mediante una terapia combinada que incluye un anticuerpo anti-CD22 o un conjugado de anticuerpo anti-CD22 con fármacos de la invención combinado con otro anticuerpo o conjugado de anticuerpo con fármaco, otro agente citotóxico, radiación u otro tratamiento administrado simultáneamente o en serie. Un método de tratamiento ejemplar de la invención es un anticuerpo anti-CD22 de la invención que se administra en combinación con un anticuerpo anti-CD20, una inmunoglobulina o un fragmento de unión a CD20 del anticuerpo, ya sea en conjunto o de manera secuencial. El anticuerpo anti-CD20 puede ser un anticuerpo desnudo o un conjugado de anticuerpo con fármacos. En una forma de realización de la terapia combinada, el anticuerpo anti-CD22 es un anticuerpo de la presente invención y el anticuerpo anti-CD20 es Rituxan® (rituximab).

El término "linfoma no Hodgkin" o "LNH", como se emplea en la presente memoria descriptiva, se refiere a un cáncer del sistema linfático que no son linfomas de Hodgkin. Los linfomas de Hodgkin se

pueden diferenciar generalmente de los linfomas no Hodgkin por la presencia de células de Reed-Sternberg en los linfomas de Hodgkin y por la ausencia de dichas células en los linfomas no Hodgkin. Los ejemplos de linfomas no Hodgkin englobados por este término según se utiliza en la presente memoria descriptiva incluyen cualquiera que un experto en la materia pudiera identificar como tal (por ejemplo un oncólogo o un patólogo) según los esquemas de clasificación conocidos en la materia, como el esquema REAL (siglas en inglés de Revised European-American Lymphoma [Linfoma revisado europeo-americano]) según se describe en el *Atlas en Color de Hematología Clínica* (3.ª edición), A. Victor Hoffbrand y John E. Pettit (eds.) (Harcourt Publishers Ltd., 2000). Véanse, en particular, las listas en las figuras 11.57, 11.58 y 11.59. Otros ejemplos más específicos incluyen, entre otros, LNH refractario o recurrente, LNH de bajo grado con tratamiento de primera línea, LNH en fase III/IV, LNH resistente a quimioterapia, leucemia y/o linfoma linfoblástico de células B precursoras, linfoma linfocítico de células pequeñas, leucemia linfocítica crónica de células B y/o leucemia prolinfocítica y/o linfoma linfocítico de células pequeñas, linfoma prolinfocítico de células B, inmunocitoma y/o linfoma linfoplasmacítico, linfoma linfoplasmacítico, linfoma de células B de zona marginal, linfoma esplénico de zona marginal, linfoma de zona marginal extranodal (MALT), linfoma de zona marginal nodal, leucemia de células pilosas, plasmacitoma y/o mieloma de células plasmáticas, linfoma folicular de bajo grado, LNH folicular de grado intermedio, linfoma de células del manto, linfoma del centro de los folículos (folicular), LNH difuso de grado intermedio, linfoma difuso de células B de gran tamaño, LNH agresivo (incluido LNH agresivo con tratamiento de primera línea y LNH agresivo recurrente), LNH recurrente después o refractario al autotrasplante de células madre, linfoma primario mediastinal de células B de gran tamaño, linfoma primario con derrame, LNH inmunoblástico de grado alto, LNH linfoblástico de grado alto, LNH de células pequeñas no divididas de grado alto, LNH voluminoso, linfoma de Burkitt, leucemia linfocítica granular amplia precursora (periférica), micosis fungoide y/o síndrome de Sezary, linfomas cutáneos, linfoma de células pequeñas anaplásicas, linfoma angiocéntrico.

En la presente memoria descriptiva, una "enfermedad autoinmune" es una enfermedad o un trastorno que se genera en, y se dirige contra, los propios tejidos u órganos de un individuo, un cosegregado o una manifestación de éste o un estado resultante de todo ello. En muchos de estos trastornos autoinmunes e inflamatorios pueden existir una serie de marcadores de laboratorio y clínicos, incluyendo, entre otros, hipergamaglobulinemia, altos niveles de autoanticuerpos, depósitos de complejos de antígeno-anticuerpo en los tejidos, beneficios de los tratamientos con corticosteroides o inmunosupresores y agregados de células linfoides en tejidos afectados. Sin limitarse a ninguna teoría relacionada con las enfermedades autoinmunes mediadas por células B, se cree que las células B demuestran un efecto patogénico en las enfermedades autoinmunes humanas mediante diversos mecanismos, incluyendo la producción de autoanticuerpos, la formación de complejos inmunes, la

activación dendrítica y de células T, la síntesis de citoquinas, la eliminación directa de quimioquinas; también se cree que estas células proporcionan un nido para la neolinfogénesis ectópica. Cada uno de estos mecanismos puede participar en diferentes grados en la patología de las enfermedades autoinmunes.

La "enfermedad autoinmune" puede ser una enfermedad de un órgano específico (es decir, la respuesta inmune se dirige específicamente contra un sistema orgánico tal como el sistema endocrino, el sistema hematopoyético, la piel, el sistema cardiopulmonar, los sistemas gastrointestinales y hepáticos, el sistema renal, las tiroides, los oídos, el sistema neuromuscular, el sistema nervioso central, etc.) o una enfermedad sistémica que puede afectar muchos sistemas orgánicos (por ejemplo, lupus sistémico eritematoso (LSE), artritis reumatoide, polimiositis, etc.). Dichas enfermedades preferentes incluyen trastornos reumatológicos autoinmunes (como por ejemplo, artritis reumatoide, síndrome de Sjögren, escleroderma, lupus tal como el LSE y el lupus nefrítico, polimiositis/dermatomiositis, crioglobulinemia, síndrome de anticuerpos antifosfolípidos y artritis psoriásica), trastornos gastrointestinales y hepáticos autoinmunes (como por ejemplo, enfermedad intestinal inflamatoria (por ejemplo, la enfermedad de Crohn y la colitis ulcerosa), gastritis autoinmune y anemia perniciosa, hepatitis autoinmune, cirrosis biliar primaria, colangitis esclerosante primaria y enfermedad celíaca), vasculitis (como por ejemplo, vasculitis ANCA negativa y vasculitis asociada a ANCA, incluyendo vasculitis de Churg-Strauss, granulomatosis de Wegener y poliangeítis microscópica), trastornos neurológicos autoinmunes (como por ejemplo, esclerosis múltiple, síndrome opsoclono-mioclono, síndrome de mioclono, miastenia gravis, neuromielitis óptica, enfermedad de Parkinson, enfermedad de Alzheimer y polineuropatías autoinmunes), trastornos renales (como por ejemplo, glomerulonefritis, síndrome de Goodpasture y enfermedad de Berger), trastornos dermatológicos autoinmunes (como por ejemplo, psoriasis, urticaria, pénfigo vulgar, penfigoide buloso y lupus eritematoso cutáneo), trastornos hematológicos (como por ejemplo, púrpura trombocitopénica, púrpura trombocitopénica trombótica, púrpura postransfusional y anemia hemolítica autoinmune), ateroesclerosis, uveítis, enfermedades hipoacúsicas autoinmunes (como por ejemplo, enfermedad del oído interno y pérdida auditiva), enfermedad de Behcet, síndrome de Raynaud, transplante de órganos, y enfermedades endocrinas autoinmunes (como por ejemplo, enfermedades autoinmunes relacionadas con la diabetes tales como la diabetes mellitus dependiente de la insulina (IDDM), enfermedad de Addison y enfermedad tiroidea autoinmune (por ejemplo, enfermedad de Graves y tiroiditis)). Las enfermedades más preferentes incluyen, por ejemplo, artritis reumatoide, colitis ulcerosa, vasculitis asociada a ANCA, lupus, esclerosis múltiple, síndrome de Sjögren, enfermedad de Graves, IDDM, anemia perniciosa, tiroiditis y glomerulonefritis.

Los ejemplos específicos de otras enfermedades autoinmunes, tal y como se definen en la presente memoria descriptiva que, en algunos casos, abarcan aquellas que se mencionaron anteriormente, incluyen,

entre otras, artritis (aguda y crónica, artritis reumatoide incluyendo artritis reumatoide de inicio juvenil y etapas tales como sinovitis reumatoide, gota o artritis gotosa, artritis inmunológica aguda, artritis inflamatoria crónica, artritis degenerativa, artritis inducida por colágeno tipo II, artritis infecciosa, artritis de Lyme, artritis proliferativa, artritis psoriásica, enfermedad de Still, artritis vertebral, osteoartritis, artritis crónica progrediente, artritis deformante, poliartritis crónica primaria, artritis reactiva, artritis menopáusica, artritis que reduce los estrógenos y espondilitis anquilosante/reumatoide), enfermedad linfoproliferativa autoinmune, enfermedades de la piel hiperproliferativas e inflamatorias, psoriasis tal como psoriasis en placa, psoriasis gutatta, psoriasis pustular y psoriasis de las uñas, atopía, incluyendo enfermedades atópicas como la fiebre del heno y el síndrome de Job, dermatitis, incluyendo dermatitis por contacto, dermatitis por contacto crónica, dermatitis exfoliativa, dermatitis alérgica, dermatitis por contacto alérgica, urticaria, dermatitis herpetiforme, dermatitis numular, dermatitis seborreica, dermatitis no específica, dermatitis por contacto irritante primaria, y dermatitis atópica, síndrome de hiper IgM ligado al x, enfermedades intraoculares alérgicas e inflamatorias, urticaria tal como urticaria alérgica crónica y urticaria idiopática crónica, incluyendo urticaria crónica autoinmune, miositis, polimiositis/dermatomiositis, dermatomiositis juvenil, necrolisis epidérmica tóxica, escleroderma (incluyendo escleroderma sistémica), esclerosis tal como esclerosis sistémica, esclerosis múltiple (EM) tal como EM espino-óptica, EM progresiva primaria (EMPP) y EM recurrente remitente (EMRR), esclerosis sistémica progresiva, aterosclerosis, arteriosclerosis, esclerosis diseminada, esclerosis atáxica, neuromielitis óptica (NMO), enfermedad intestinal inflamatoria (EII) (por ejemplo, enfermedad de Crohn, enfermedades intestinales de origen autoinmune, inflamación gastrointestinal, colitis como colitis ulcerativa, colitis ulcerosa, colitis microscópica, colitis colagenosa, colitis poliposa, enterocolitis necrotizante y colitis transmural, y enfermedad intestinal inflamatoria autoinmune), inflamación intestinal, piodeṛma gangrenoso, eritema nodoso, colangitis esclerosante primaria, síndrome de trastorno respiratorio, incluyendo síndrome de trastorno respiratorio adulto o agudo (STRAA), meningitis, inflamación de toda o parte de la úvea, iritis, coroiditis, un trastorno hematológico autoinmune, enfermedades de injerto contra huésped, angioedema tal como angioedema hereditario, daño del nervio craneal como en el caso de la meningitis, herpes gestationis, penfigoide gestationis, prurito de escroto, insuficiencia ovárica prematura autoinmune, hipoacusia súbita debido a una afección autoinmune, enfermedades mediadas por IgE tales como anafilaxia y rinitis alérgica y atópica, encefalitis como encefalitis de Rasmussen, encefalitis límbica y/o encefálica, uveítis, como uveítis anterior, uveítis anterior aguda, uveítis granulomatosa, uveítis no granulomatosa, uveítis facoantigénica, uveítis posterior o uveítis autoinmune, glomerulonefritis (GN) con y sin síndrome nefrótico como glomerulonefritis crónica o aguda tal como GN primaria, GN de origen inmune, GN membranosa (nefropatía membranosa), GN membranosa idiopática o nefropatía membranosa idiopática, GN de membrana o membranosa proliferativa (GNMP), incluyendo Tipo I y Tipo II, y GN

progresiva rápida (GNPR), nefritis proliferativa, insuficiencia poliglandular endocrina autoinmune, balanitis incluyendo balanitis circunscrita plasmocelular, balanopostitis, eritema anular centrífuga, eritema discrómico perstans, eritema multiforme, granuloma anular, liquen nitidus, liquen escleroso y atrófico, liquen simple crónico, liquen espinuloso, liquen plano, ictiosis laminar, hiperqueratosis epidermolítica, queratosis premaligna, pioderma gangrenosa, afecciones y respuestas alérgicas, alergias a los alimentos, alergias a los fármacos, alergias a los insectos, enfermedades alérgicas poco frecuentes tales como mastocitosis, reacción alérgica, eccema, incluyendo eccema alérgico o atópico, eccema asteatótico, eccema dishidrótico y eccema vesicular palmoplantar, asma tal como asma bronquial y asma autoinmune, afecciones que implican infiltración de células T y respuestas inflamatorias crónicas, reacciones inmunes contra antígenos extraños tales como grupos sanguíneos fetales A-B-O durante el embarazo, enfermedad inflamatoria pulmonar crónica, miocarditis autoinmune, deficiencia de adhesión leucocitaria, lupus, incluyendo nefritis, cerebritis, lupus pediátrico, lupus no renal, lupus extra renal, lupus discoide y lupus discoide eritematoso, alopecia, LSE tal como LSE cutáneo o LSE cutáneo subagudo, síndrome de lupus neonatal (SLN) y lupus eritematoso diseminado, diabetes mellitus de inicio juvenil (Tipo I), incluyendo IDDM pediátrico, diabetes mellitus de inicio adulto (diabetes Tipo II), diabetes autoinmune, diabetes insípida idiopática, retinopatía diabética, nefropatía diabética, colitis diabética, alteración de las grandes arterias diabética, respuestas inmunes asociadas a hipersensibilidad aguda y retardada mediada por citocinas y linfocitos T, tuberculosis, sarcoidosis, granulomatosis incluyendo granulomatosis linfomatoide, agranulocitosis, vasculitis (incluyendo vasculitis de grandes vasos tal como polimialgia reumática y células gigantes (artritis de Takayasu arteritis), vasculitis de vasos medianos tal como enfermedad de Kawasaki y poliarteritis nodosa/periarteritis nodosa, ovasculitis inmune, vasculitis del SNC, vasculitis cutánea, vasculitis por hipersensibilidad, vasculitis necrotizante tal como vasculitis necrotizante fibrinoide y vasculitis necrotizante sistémica, vasculitis ANCA negativa y vasculitis asociada a ANCA tal como el síndrome de Churg-Strauss (SCS), granulomatosis de Wegener y poliangiitis microscópica), arteritis temporal, anemia aplásica, anemia aplásica autoinmune, anemia positiva de Coombs, anemia de Diamond Blackfan, anemia hemolítica o anemia hemolítica inmune, incluyendo anemia hemolítica autoinmune (AIHA), anemia perniciosa, enfermedad de Addison, la aplasia de glóbulos rojos puros (PRCA), deficiencia del factor VIII, hemofilia A, neutropenia autoinmune, citopenias tales como pancitopenia, leucopenia, enfermedades relacionadas con diapédesis leucocitaria, trastorno inflamatorio del SNC, enfermedad de Alzheimer, enfermedad de Parkinson, síndrome de lesiones de múltiples órganos como los secundarios a septicemias, traumas o hemorragias, enfermedades mediadas por complejos antígena-anticuerpo, enfermedad de membrana basal antiglomerular, síndrome de anticuerpos antifosfolípidos, motoneuritis, neuritis alérgica, síndrome/enfermedad de Behçet, síndrome de Castleman, síndrome de Goodpasture, síndrome de Reynaud, síndrome de Sjögren, síndrome de Stevens-Johnson, penfigoide tal

como penfigoide buloso, penfigoide cicatricial (membrana mucosa), penfigoide dérmico, penfigoide vulgar, penfigoide paraneoplásico, penfigoide foliáceo, pénfigo de membrana mucosa penfigoide y pénfigo eritermatoso, epidermólisis bulosa adquirida, inflamación ocular, inflamación ocular preferentemente alérgica tal como conjuntivitis alérgica, enfermedad bulosa IgA linear, inflamación conjuntival inducida y autoinmune, poliendocrinopatías autoinmunes, enfermedad o síndrome de Reiter, lesión térmica debido a una afección autoinmune, preclampsia, trastorno por inmunocomplejos tal como nefritis por inmunocomplejo, nefritis mediada por anticuerpos, trastornos neuroinflamatorios, polineuropatías, neuropatía crónica tal como polineuropatías IgM o neuropatías con mediación de IgM, trombocitopenia (tal como la desarrollan los pacientes con infarto de miocardio, por ejemplo), incluyendo púrpura trombótico trombocitopánico (TTP), púrpura postransfusional (PTF), trombocitopenia inducida por heparina y trombocitopenias inmunes y autoinmunes incluyendo, por ejemplo, púrpura trombocitopánica idiopática (PTI) incluida PTI crónica o aguda, escleritis tal como queratoescleritis idiopática, episcleritis, enfermedad autoinmune de los testículos y ovarios, incluyendo orquitis y ooforitis autoinmunes, hipotiroidismo primario, hipoparatiroidismo, enfermedades endocrinas autoinmunes, incluyendo tiroiditis tal como tiroiditis autoinmune, enfermedad de Hashimoto, tiroiditis crónica (tiroiditis de Hashimoto) o tiroiditis subaguda, enfermedad de tiroides autonmune, hipotiroidismo idiopático, enfermedad de Grave, enfermedad ocular de Grave (oftalmopatía u oftalmopatía asociada a las tiroides), síndromes poliglandulares tales como síndromes poliglandulares autoinmunes, por ejemplo, tipo I (o síndromes poliglandulares endocrinopáticos), síndromes paraneoplásicos, incluyendo síndromes paraneoplásicos neurológicos tales como síndrome miasténico de Lambert-Eaton o síndrome de Eaton-Lambert, síndrome de rigidez muscular, encefalomielitis tal como encefalomielitis alérgica o encefalomielitis alérgica experimental (EAE), miastenia gravis tal como miastenia gravis asociada a timoma, degeneración cerebelar, neuromiotonía, opsoclono o síndrome de opsoclono mioclono (SOM) y neuropatía sensorial, neuropatía motora multifocal, síndrome de Sheehan, hepatitis autoinmune, hepatitis crónica, hepatitis lupoide, hepatitis de células gigantes, hepatitis activa crónica o hepatitis activa crónica autoinmune, neumonitis tal como neumonitis intersticial linfoide (NIL), bronquiolitis obliterante (sin transplante) frente a NSIP, síndrome de Guillain-Barré, enfermedad de Berger (nefropatía por IgA), nefropatía por IgA idiopática, dermatosis por IgA linear, dermatosis neutrofílica febril aguda, dermatosis pustular subcorneal, dermatosis acantolítica transitoria, cirrosis tal como cirrosis biliar primaria y neumonocirrosis, síndrome enteropático autoinmune, enfermedad celíaca o coelíaca, esprue celíaco (enteropatía por gluten), esprue refractario, esprue idiopático, crioglobulinemia tal como crioglobulinemia mixta, esclerosis lateral amilotrófica (ELA; enfermedad de Lou Gehrig), enfermedad arterial coronaria, enfermedad del oído autoinmune tal como enfermedad del oído interno autoinmune (EOIA), pérdida auditiva autoinmune, policondritis como policondritis refractaria o recurrente, proteinosis alveolar pulmonar, queratitis tal como

síndrome de Cogan/queratitis intersticial no sifilítica, parálisis de Bell, enfermedad/síndrome de Sweet, rosácea autoinmune, dolor asociado al zóster, amiloidosis, linfocitosis no cancerígena, linfocitosis primaria, que incluye linfocitosis de las células B monoclonales (por ejemplo, gamapatía monoclonal benigna y gamapatía monoclonal de significancia indeterminada, MGUS), neuropatía periférica, síndrome paraneoplásico, canelopatías tales como epilepsia, migraña, arritmia, trastornos musculares, sordera, ceguera, parálisis periódica y canelopatías del SNC, autismo, miopatía inflamatoria, focal o segmental, o glomeruloesclerosis focal segmental (GEFS), oftalmopatía endocrina, uveorretinitis, coriorretinitis, trastorno hepatológico autoinmune, fibromialgia, insuficiencia endocrina múltiple, síndrome de Schmidt, adrenalitis, atrofia gástrica, demencia presenil, enfermedades de desmielinización tales como enfermedades desmielinizantes y autoinmunes y polineuropatía desmielinizante inflamatoria crónica, síndrome de Dressler, alopecia areata, alopecia total, síndrome de CREST (calcinosis, fenómeno de Raynaud, dismotilidad esofágica, esclerodactilia y telangiectasia), esterilidad masculina y femenina autoinmune debido a, por ejemplo, anticuerpos antiespermatozoides, enfermedad mixta del tejido conjuntivo, enfermedad de Chagas, fiebre reumática, aborto recurrente, pulmón del granjero, eritema multiforme, síndrome de poscardiotomía, síndrome de Cushing, pulmón del cuidador de aves, angeítis granulomatosa alérgica, angeítis linfocítica benigna, síndrome de Alport, alveolitis como alveolitis alérgica y alveolitis fibrosa, enfermedad pulmonar interstitial, reacciones de transfusión, lepra, malaria, enfermedades parásiticas tales como leishmaniasis, quipanosomiasis, esquistosomiasis, ascariasis, aspergilosis, síndrome de Sampter, síndrome de Caplan, dengue, endocarditis, fibrosis endomiocárdiaca, fibrosis pulmonar intersticial difusa, fibrosis pulmonar intersticial, mediastinitos fibrosa, fibrosis pulmonar, fibrosis pulmonar idiopática, fibrosis cística, endoftalmitis, eritema elevado diutinum, eritroblastosis fetal, fascitis eosinófila, síndrome de Shulman, síndrome de Felty, flariasis, ciclitis, como la ciclitis crónica, la ciclitis heterocrónica, iridociclitis (aguda o crónica) o ciclitis de Fuch, púrpura de Henoch-Schonlein, infección por el virus de inmunodeficiencia humana (VIH), SCID, síndrome de inmunodeficiencia adquirida (SIDA), infección por ecovirus, sepsis (síndrome de respuesta inflamatoria sistémica (SRIS)), endotoxemia, pancreatitis, tiroxicosis, infección por parvovirus, infección por el virus de la rubéola, síndromes de posvacunación, infección por rubéola congénita, infección por el virus de Epstein-Barr, paperas, síndrome de Evan, disfunción gonadal autoinmune, corea de Sydenham, nefritis posestreptocócica, tromboangitis obliterante, tirotoxicosis, tabes dorsal, corioiditis, polimialgia de células gigantes, neumonitis hipersensible crónica, conjuntivitis, tal como catarro vernal, queratoconjuntivitis seca y queratoconjuntivitis epidémica, síndrome nefrítico idiopático, nefropatía de cambio mínimo, lesión por reperfusión isquémica benigna familiar, reperfusión de órganos transplantados, autoinmunidad retiniana, inflamación de las articulaciones, bronquitis, enfermedad respiratoria obstructiva crónica, silicosis, afta, estomatitis aftosa, trastornos arterioescleróticos (insuficiencia cerebrovascular) tal como encefalopatía

arteriosclerótica y retinopatía arteriosclerótica, aspermiogénesis, hemolisis autoinmune, enfermedad de Boeck, crioglobulinemia, contractura de Dupuytren, endoftalmia facoanafiláctica, enteritis alérgica, eritema nodoso leproso, parálisis facial idiopática, síndrome de fatiga crónica, fiebre reumática, enfermedad de Hamman-Rich, pérdida auditiva sensoneural, hemoglobinuria paroxismática, hipogonadismo, ileítis regional, leucopenia, mononucleosis infecciosa, mielitis transversa, mixedema idiopático primario, nefrosis, oftalmia simpática, oftalmitis neonatal, neuritis óptica, orquitis granulomatosa, pancreatitis, polirradiculitis aguda, pioderma gangrenoso, tiroiditis de Quervain, atrofia esplénica adquirida, timoma no maligno, timitis linfofolicular, vitiligo, índrome de choque tóxico, intoxicación alimentaria, afecciones que implican infiltración de células T, deficiencia de adhesión leucocitaria, respuestas inmunes asociadas con hipersensibilidad aguda y recurrente mediada por citoquinas y linfocitos T, enfermedades que implican diapédesis leucocitaria, síndrome de lesión orgánica múltiple, enfermedades mediadas por complejos antígeno-anticuerpo, enfermedad de la membrana basal antiglomerular, poliendocrinopatías autoinmunes, ooforitis, mixedema primario, gastritis atrófica autoinmune, enfermedades reumáticas, enfermedad del tejido conectivo mixta, síndrome nefrótico, insulitis, insuficiencia poliendocrina, síndrome poliglandular autoinmune, incluyendo síndrome poliglandular tipo I, hipoparatiroidismo de inicio en adultos idiopático (HIAI), cardiomiopatía como cardiomiopatía dilatada, epidermolisis bullosa adquirida (EBA), hemocromatosis, miocarditis, síndrome nefrótico, colangitis esclerosante primaria, sinusitis purulenta o no purulenta, sinusitis aguda o crónica, sinusitis etmoidea, frontal, maxilar o esfenoidea, sinusitis alérgica, trastorno relacionado con los eosinófilos como eosinofilia, eosinofilia por infiltración pulmonar, síndrome de eosinofilia-mialgia, síndrome de Loffler, neumonía eosinofílica crónica, eosinofilia pulmonar tropical, aspergilosis bronconeumónica, aspergiloma o granulomas con eosinófilos, anafilaxis, espondiloartropatía, espondiloartritis seronegativa, enfermedad autoinmune poliendocrina, colangitis esclerosante, esclera, episclera, candidiasis mucocutánea crónica, síndrome de Bruton, hipogammaglobulinemia temporal de la infancia, síndrome de Wiskott-Aldrich, ataxia telangiectasia, angiectasias, trastornos autoinmunes asociados con enfermedades del colágeno, reumatismo como artroreumatismo crónico, linfadenitis, reducción de la respuesta a la presión sanguínea, disfunción vascular, daño tisular, isquemia cardiovascular, hiperalgesia, isquemia renal, isquemia cerebral y enfermedad que acompaña a la vascularización, trastornos por hipersensibilidad alérgica, glomerulonefritis, lesión por reperfusión, trastorno por reperfusión siquémica, lesión por reperfusión del miocardio u otros tejidos, traqueobronquitis linfomatosa, dermatosis inflamatoria, dermatosis con componentes inflamatorios agudos, fallo multiorgánico, enfermedades bulosas, necrosis cortical renal, meningitis purulenta aguda u otros trastornos inflamatorios del sistema nervioso central, trastornos inflamatorios oculares y orbitales, síndromes granulocíticos asociados a la transfusión, toxicidad inducida por citoquinas, narcolepsia, inflamación grave

aguda, inflamación crónica sin tratamiento, pielitis, hiperplasia endarterial, úlcera péptica, valvulitis y endometriosis. Estas enfermedades que se consideran en la presente memoria descriptiva se tratan mediante la administración de un anticuerpo que se une a un marcador de la superficie de células B tal como el CD22 e incluye la administración de un anticuerpo no conjugado ("desnudo") o un anticuerpo conjugado con un agente citotóxico, tal como se describe en la presente memoria descriptiva. Las enfermedades que se consideran en la presente memoria descriptiva también se tratan mediante una terapia combinada que incluye un anticuerpo anti-CD22 o un conjugado de anticuerpo anti-CD22 con fármacos de la invención combinado con otro anticuerpo o conjugado de anticuerpo con fármaco, otro agente citotóxico, radiación u otro tratamiento administrado simultáneamente o en serie.

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.

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.

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.

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.

Una "toxina" es una sustancia con capacidad para tener un efecto perjudicial en el crecimiento o proliferación de una célula.

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 etileniminas 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 mecloretamina, 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 1I (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 mepitiiostano, 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

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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 (ELOXATINTM) combinado con 5-FU y leucovovina.

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.

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 CD22) 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 CD22) 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.

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.

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.

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.

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.

"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.

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.

"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)

"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),

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.

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.

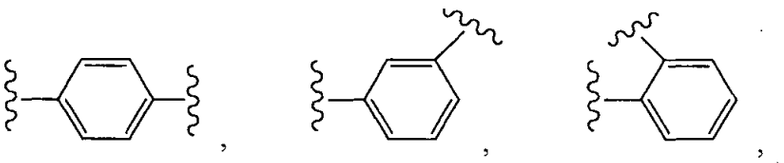
Por "alquenileno" se entiende un radical hidrocarbono insaturado, cíclico o de cadena recta o ramificada de 2 a 18 átomos de carbono, con dos centros radicales monovalentes derivados mediante la eliminación de dos átomos de hidrógeno del mismo átomo o de dos átomos de carbono distintos de un alqueno padre. Entre los radicales alquenilenos típicos, están: 1,2-etileno (-CH=CH-).

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-).

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)$_2$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.

Un "amileno" 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)$_2$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.

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.

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].

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, -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.

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].

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 hasta hoy), en concreto en los Volúmenes 13, 14, 16, 19 y 28; y *J. Am. Chem. Soc.* (1960) 82:5566.

Ejemplos non-limitantes 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.

A modo de ejemplo non-limitante, 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.

A modo de ejemplo non-limitante, 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.

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.

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.

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 monocíclicos 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.

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', -$S(O)_2R'$, -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.

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.

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.

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.

Por "estereoisomeros" se entienden los compuestos que tienen idéntica constitución química pero difieren en la disposición de los átomos o grupos en el espacio.

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.

Por "enantiomeros" se entiende dos estereoisomeros de un compuesto que no se superponen como contraimagen el uno del otro.

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.

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

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:

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

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

Se proporcionan anticuerpos que se unen a CD22. Se proporcionan inmunoconjugados que comprenden anticuerpos anti-CD22. 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 CD22. 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-CD22

En un aspecto, la invención proporciona anticuerpos que se unen a CD22. En algunas formas de realización, se proporcionan anticuerpos que se unen a una forma madura del CD22 de humanos y monos cinomolgus. En una de estas formas de realización, una forma madura del CD22 humano tiene una secuencia de aminoácidos de identificador de secuencia n.º: 27. La isoforma humana madura principal tiene un dominio extracelular que comprende siete doiminios de tipo Ig y una secuencia de aminoácidos de identificador de secuencia n.º: 28. En otra forma de realización, una isoforma secundaria de CD22 humana que carece de los dominios extracelulares 3 y 4 tiene una secuencia de aminoácidos de identificador de secuencia n.º: 29. La secuencia de aminoácidos del dominio extracelular de la isoforma secundaria es el identificador de secuencia n.º: 30. El CD22 cinomolgus tiene una secuencia de aminoácidos de identificador de secuencia n.º: 31. En algunas formas de realización, un anticuerpo de CD22 se une a una forma madura de CD22 expresado en una superficie celular. En algunas formas de realización, un anticuerpo que se une a una forma madura de CD22 expresada en la superficie celular inhibe el crecimiento de la célula. En algunas formas de realización, un anticuerpo anti-CD22 se une a una forma madura de CD22 expresada en la superficie celular e inhibe la proliferación celular. En algunas formas de realización, un anticuerpo anti-CD22 se une a una forma madura de CD22 expresada en la superficie celular e induce la muerte celular. En algunas formas de realización, un anticuerpo anti-CD22 se une a una forma madura de CD22 expresada en la superficie de células cancerosas. En algunas formas de realización, un anticuerpo anti-CD22 se una a una forma madura de CD22 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-CD22 se conjuga a una citotoxina o a una marca detectable y se une al CD22 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 CD22 en su superficie para que sea detectable *in vitro* o *in vivo*.

En un aspecto, un anticuerpo anti-CD22 es un anticuerpo monoclonal. En un aspecto, un anticuerpo anti-CD22 es un fragmento de anticuerpo, por ejemplo, un fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$. En un aspecto, un anticuerpo anti-CD22 es un anticuerpo quimérico, humanizado o humano. En un aspecto, cualquiera de los anticuerpos anti-CD22 descritos en la presente memoria descriptiva está purificado.

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 CD22 beta y alfa. Los anticuerpos que se obtienen de la detección de la biblioteca tienen una maduración de afinidad.

En un aspecto, se proporcionan anticuerpos monoclonales que compiten con el anticuerpo murino 10F4.4.1, el humanizado 10F4v1 y v3, y el murino 5E8.1.8 para que se unan al CD22. También se proporcionan anticuerpos monoclonales que se unen al mismo epítopo como el anticuerpo murino 10F4.4.1, el humanizado 10F4v1 y v3, y el murino 5E8.1.8.

En un aspecto de la invención, se proporcionan polinucleótidos que codifican anticuerpos anti-CD22. En ciertas formas de realización, se proporcionan vectores que comprenden polinucleótidos que codifican anticuerpos anti-CD22. En ciertas formas de realización, se proporcionan células huésped que comprenden estos vectores. En otro aspecto de la invención, se proporcionan composiciones que comprenden anticuerpos anti-CD22 o polinucleótidos que codifican anticuerpos anti-CD22. 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.

Administración y formulación de los anticuerpos

En una forma de realización, el anticuerpo anti-CD22 o el conjugado de anticuerpo anti-CD22 con fármacos (incluyendo, entre otros, el conjugado de thiomab anti-CD22 de la invención con fármacos) de la

invención se administra en combinación con un antagonista de un antígeno de la superificie de células B. La administración "en combinación con" uno o más agentes terapéuticos incluye la administración simultánea (concurrente) y consecutiva en cualquier orden. En una forma de realización, la administración es consecutiva o secuencial. En otra forma de realización, la administración es simultánea, concurrente o conjunta en la misma formulación. En una forma de realización, el antagonista del antígeno de la superficie de células B es un anticuerpo o un antígeno que une fragmentos de éstos. En una forma de realización, el antagonista de la superficie de células B es un conjugado de anticuerpo con fármacos.

Las formulaciones de la presente memoria descriptiva pueden contener más de un compuesto activo tal como sea necesario para la indicación particular que se esté tratando, preferentemente aquellas con actividades complementarias que no se afectan de manera adversa entre sí. Por ejemplo, además de un anticuerpo anti-CD22, un conjugado de anticuerpo anti-CD22 con fármacos o un oligopéptido de unión CD22, puede que sea deseable incluir en la formulación un anticuerpo adicional, como por ejemplo, un segundo anticuerpo anti-CD22 que une un epítopo diferente en el polipéptido CD22, un segundo anticuerpo que une un antígeno diferente de la superficie de células B o un anticuerpo de alguna otra diana tal como un factor de crecimiento que afecta el crecimiento de un cáncer determinado. Alternativa o adicionalmente, la composición puede comprender 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.

Actualmente, según la fase del cáncer, el tratamiento contra el cáncer implica uno o una combinación de las siguientes terapias: cirugía para eliminar el tejido canceroso, radioterapia y quimioterapia. El anticuerpo anti-CD22, el conjugado de anticuerpo anti-CD22 con fármacos o la terapia oligopéptida pueden aplicarse especialmente en pacientes ancianos que no toleran bien la toxicidad ni los efectos secundarios de la quimioterapia, y en los pacientes que padecen enfermedades metastáticas en las que la radioterapia tiene poca utilidad. Los anticuerpos anti-CD22, los conjugados de anticuerpo anti-CD22 con fármacos o los oligopéptidos de la invención que actúan sobre el tumor son útiles para aliviar los cánceres que expresan CD22 tras el diagnóstico inicial de la enfermedad o durante la recidiva. Para las aplicaciones terapéuticas, el anticuerpo anti-CD22, el conjugado de anticuerpo anti-CD22 con fármacos o el oligopéptido pueden utilizarse por separado o en una terapia combinada con, por ejemplo, hormonas, antiangiógenes o compuestos radiomarcados, o con cirugía, crioterapia o radioterapia, o todas las anteriores. El tratamiento con anticuerpos anti-CD22, conjugados de anticuerpo anti-CD22 con fármacos o oligopéptidos puede administrarse en combinación con otras formas de terapia convencional, de manera consecutiva ya sea con la terapia preconvencional o la posconvencional. En el presente método de la

invención para tratar o aliviar el cáncer, se le puede administrar al paciente con cáncer un anticuerpo anti-CD22, un conjugado de anticuerpo anti-CD22 con fármacos o un oligopéptido en combinación con un tratamiento con uno o más de los siguientes agentes quimioterapéuticos. El anticuerpo anti-CD22, el conjugado de anticuerpo anti-CD22 con fármacos o el oligopéptido se administrarán con una dosis terapéuticamente eficaz de un agente quimioterapéutico. En otra forma de realización, el anticuerpo anti-CD22, el conjugado de anticuerpo anti-CD22 con fármacos o el oligopéptido se administran en combinación con la quimioterapia para mejorar la actividad y eficacia del agente quimioterapéutico. Las dosis detalladas en la Referencia de escritorio de los médicos (PDR, por sus siglas en inglés) de estos agentes se han utilizado en el tratamiento de diversos cánceres. La pauta posológica de estos fármacos quimioterapéuticos mencionados anteriormente que son terapéuticamente eficaces dependerá del cáncer en particular que deba tratarse, el grado de la enfermedad y otros factores conocidos por los médicos expertos en la materia, y el médico puede determinar dicho régimen.

En una forma de realización, se administra al paciente un conjugado que comprende un anticuerpo anti-CD22, un conjugado de anticuerpo anti-CD22 con fármacos o un oligopéptido conjugados con un agente citotóxico. Preferentemente, la célula internaliza el inmunoconjugado unido a la proteína CD22 lo que incrementa la eficacia terapéutica del inmunoconjugado para matar célula cancerosa a la que dicho inmunoconjugado se une. En una forma de realización, el agente citotóxico actúa o interfiere con el ácido nucleico en la célula cancerosa. Los ejemplos de agentes citotóxicos se describen anteriormente e incluyen auristatinas, maitansinoides, caliqueamicinas, ribonucleasas y ADN con endonucleasas, o sus derivados biológicamente activos.

Los anticuerpos anti-CD22, los conjugados de anticuerpo anti-CD22 con fármacos, los oligopéptido o sus conjugados con toxinas se administran al paciente humano de acuerdo con los procedimientos conocidos, como por ejemplo, la administración intravenosa, como un bolo o una infusión continua durante un período de tiempo, mediante vías intramusculares, intraperitoneales, cefalorraquídeas, subcutáneas, intraarticulares, intrasinoviales, intratecales, orales, tópicas o por inhalación. Se prefiere la administración intravenosa o subcutánea de un anticuerpo, un conjugado de anticuerpo anti-CD22 con fármacos o un oligopéptido.

La administración de un anticuerpo, un conjugado de anticuerpo anti-CD22 con fármacos o un oligopéptido puede combinarse con otros regímenes terapéuticos. La administración combinada incluye la coadministración, utilizando formulaciones separadas o una única formulación farmacéutica, y la administración consecutiva en cualquier orden, en el que preferentemente exista un período en el cual

ambos (o todos los) agentes activos ejerzan simultáneamente sus actividades biológicas. Preferiblemente, este tratamiento combinado desencadena un efecto terapéutico sinérgico.

También puede combinarse, según se desee, la administración de un anticuerpo o anticuerpos anti-CD22, conjugados de anticuerpo anti-CD22 con fármacos u oligopéptidos con la administración de un anticuerpo dirigido contra otro antígeno tumoral o antígeno de la superficie de células B asociado a un cáncer determinado.

En otra forma de realización, los métodos de tratamiento terapéutico de la presente invención incluyen la administración combinada de anticuerpo/s anti-CD22, conjugado/s de anticuerpo anti-CD22 con fármacos o oligopéptido/s y uno o más agentes quimioterapéuticos o agentes inhibidores del crecimiento, incluyendo la coadministración de un cóctel de diferentes agentes quimioterapéuticos. Los agentes quimioterapéuticos incluyen fosfato de estramustina, prednimustina, cisplatino, 5-fluorouracilo, melfalán, ciclofosfamida, hidroxiurea e hidroxiureataxanos (como por ejemplo, paclitaxel y doxetaxel) o antibióticos a base de antraciclina, o todos los anteriores, así como combinaciones de agentes tales como CHOP o FOLFOX, entre otros. Los calendarios de preparación y dosificación para dichos agentes quimioterapéuticos se pueden utilizar de acuerdo a las instrucciones del fabricante y tal y como lo determine empíricamente el médico cualificado. Los calendarios de preparación y dosificación para esta quimioterapia se describen también en *Chemotherapy Service* Ed., M.C. Perry, Williams y Wilkins, Baltimore, MD (1992).

El anticuerpo se administra por cualquier medio apto, incluyendo las vías parenteral, tópica, subcutánea, intraperitoneal, intrapulmonar, intranasal o intralesional, o todas las anteriores. Las infusiones parentales incluyen la administración intramuscular, intravenosa, intraarterial, intraperitoneal y subcutánea. La administración intratecal también se contempla (véase, por ejemplo, la solicitud de patente de EE.UU. N.° 2002/0009444, Grillo-López, A., referente a la introducción intratecal de un anticuerpo CD20). Preferentemente, la dosis se administra por vía intravenosa o subcutánea.

Un segundo medicamento puede administrarse con la exposición inicial o las exposiciones posteriores, o ambas, del anticuerpo terapéutico o la inmunoadhesina, dicha administración combinada incluye la coadministración, utilizando formulaciones separadas o una única formulación farmacéutica, y la administración consecutiva en cualquier orden, en que preferentemente existe un período en el cual ambos (o todos los) agentes activos ejercen simultáneamente sus actividades biológicas.

Si bien el anticuerpo anti-CD22 terapéutico, el conjugado de anticuerpo anti-CD22 con fármacos, la inmunoadhesina u otro fármaco biológico pueden administrarse como un único agente para tratar la

enfermedad autoinmune, por lo general, el anticuerpo terapéutico o la inmunoadhesina se combinarán con uno o más medicamentos secundarios. Por ejemplo, en el caso de la artritis reumatoide y otras enfermedades autoinmunes, el anticuerpo, la inmunoahesina u otro fármaco biológico se combinan preferentemente con uno o más de los agentes inmunosupresores, los agentes quimiterapéuticos, los antagonistas del BAFF, los antagonistas o anticuerpos integrina o las citoquinas, o todos los anteriores, que se enumeran en la sección de definiciones mencionada anteriormente; uno o más de los fármacos antirreumáticos moduladores de la enfermedad (DMARD), como por ejemplo, hidroxicloroquina, sulfasalazina, metotrexato, leflunomida, azatioprina, D-penicilamina, Gold (oral), Gold (intramuscular), minociclina, ciclosporina; inmunoabsorción con proteína A estafilocócica; inmunoglobulina intravenosa (IGIV); medicamentos antiinflamatorios no esteroides (NSAID); glucocorticoide (por ejemplo, a través de una inyección en la articulación); corticosteroide (por ejemplo, metilprednisolona o prednisona); folato; antagonista del factor de necrosis tumoral (TNF), por ejemplo, etanercept/ENBREL™, infliximab/REMICADE™, D2E7 (Knoll) o CDP-870 (Celltech); un antagonista IL-1R (por ejemplo, Kineret); un antagonista IL-10 (por ejemplo, Ilodecakin); un modulador de coagulación sanguínea (por ejemplo, WinRho); un antagonista IL-6/anti-TNF (CBP 1011); un antagonista CD40 (por ejemplo, IDEC 131); antagonista del receptor de Ig-Fc (MDX33); inmunomodulator (por ejemplo, talidomida o ImmuDyn); un anticuerpo anti-CD5 (por ejemplo, H5g1.1); un inhibidor de macrófagos (por ejemplo, MDX 33); un bloqueante coestimulador (por ejemplo, BMS 188667 o Tolerimab); un inhibidor del complemento (por ejemplo, h5G1.1, 3E10 o un anticuerpo del factor de aceleración del decaimiento (DAF)); un antagonista IL-2 (zxSMART); un inhibidor de EGFR (véase la definición anterior); un inhibidor de tirosina quinas (véase definición anterior); un agente anti-angiogénico (por ejemplo, anticuerpo VEGF tal como bevacizumab); anticuerpos CD22 tales como LL2 o epratuzumab (LYMPHOCIDE®; Immunomedics), incluyendo epratuzumab Y-90 (Juweid y col., *Cancer Res* 55(23 Suppl):5899s-5907s (1995)), un anticuerpo CD22 de Abiogen (Abiogen, Italia), CMC 544 (Wyeth/Celltech), combotox (UT Soutwestern), BL22 (NIH) y LympoScan Tc99 (Immunomedics); anticuerpo EpCAM tal como 17-1A (PANOREX®); anticuerpo ∀v∃3 (por ejemplo, VITAXIN®; Medimmune); anticuerpo CD37 tal como TRU 016 (Trubion); anticuerpo IL-21 (Zymogenetics/Novo Nordisk); anticuerpo anti-células B (Impheron); MAb que actúa sobre las células B (Immunogen/Aventis); 1D09C3 (Morphosys/GPC); LymphoRad 131 (HGS); anticuerpo Y-90 Lym-1 (USC); LIF 226 (Enhanced Lifesci); anticuerpo BAFF (por ejemplo, documento WO 03/33658); anticuerpo del receptor de BAFF (por ejemplo, documento WO 02/24909); anticuerpo BR3; anticuerpo Blys tal como belimumab; LYMPHOSCD22 -B™; Oncolym anti-Lym-1 (USC/Peregrine); ISF 154 (UCSD/Roche/Tragen); gomiliximа (Idec 152; Biogen Idec); anticuerpo del receptor de IL-6 tal como atlizumab (ACTEMRA™; Chugai/Roche); anticuerpo IL-15 tal como HuMax-Il-15 (Genmab/Amgen); anticuerpo del receptor de la

quimioquina tal como un anticuerpo CCR2 (por ejemplo, MLN1202; Millieneum); anticuerpo anticomplemento tal como el anticuerpo C5 (por ejemplo, eculizumab, 5G1.1; Alexion); formulación oral de inmunoglobulina humana (por ejemplo, IgPO; Protein Therapeutics); anticuerpo IL-12 tal como ABT-874 (CAT/Abbott); Teneliximab (BMS-224818); vacuna de células B; DN-BAFF (Xencor); CRx-119 (CombinatoRx); antagonista del BAFF de Amgen; pentostatina (Pfizer); IC-485 (ICOS); antagonista de la quimioquina tal como T-487 (Tularik) o reticulosa (AVR-118); SCO-323 (SCIOS); antagonista de la integrina 683699, Tanabe, NGD-2001-1 (Neurogen); SCIO-469 (SCIOS); BIRB-796 (Boehringer Ingelheim); VX702, VX850 (Vertex); antagonista de leucotrieno B-4 (tal como amelubunt, BIIL-284; BI); modulador de microtúbulos (Paxceed; Angiotech); inhibidor de la proteasa (MBS561392; BMS); AGIX-4207 (Atherogenics); ISIS-104838 (ISIS/Elan); MFG-IRAP (Univ. Pitt.); IL-1 Trap (RGN-303; Regeneron/Novartis); oprelvequin (Wyeth); everolimo (Certican; Novartis); Amevive (Biogen Idec); ORG-39141 (Organon); FK-506 (Fujisawa); y un antagonista de IL-2 (tacrolimo; Fujisawa).

A continuación se incluye una descripción detallada de anticuerpos anti-CD22 ejemplares:

1. Formas de realización específicas de anticuerpos anti-CD22

En un aspecto, la invención proporciona un anticuerpo que comprende al menos una, dos, tres, cuatro, cinco o seis regiones hipervariables seleccionadas de (a) una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 2; (b) una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 4; (c) una HVR-H3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 6; (d) una HVR-L1 que comprende la secuencia de aminoácidos de cualquiera de los identificadores de secuencia n.º 9, 10, 19, 20, 21, 22, 23; (e) una HVR-L2 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 12 y (f) una HVR-L3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 14.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende al menos una, dos o las tres secuencias de la HVR de VH seleccionadas de (a) una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 2; (b) una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 4; (c) una HVR-H3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 6. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 2. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n.º: 4. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 6.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 6 y una HVR-H1 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 2.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 6 y una HVR-H2 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 4.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 2 y una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 4.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 2 y una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 4 y una HVR-H3 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 6.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende al menos una, al menos dos o todas las tres secuencias HVR de VL seleccionadas de (a) una HVR-L1 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 o del identificador de secuencia n.°: 10; (b) una HVR-L2 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 12 y (c) una HVR-L3 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 14. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-L1 que comprende una secuencia de aminoácidos seleccionada del identicador de secuencia n.° 9. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-L1 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 10. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-L1 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 19-23. En un aspecto, la HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que V sustituye a N28 (cambio del aminoácido N28V, que genera el identificador de secuencia n.°: 10). En un aspecto, la HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que A sustituye a N28 (cambio del aminoácido N28A, que genera el identificador de secuencia n.°: 19). En un aspecto, la HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que Q sustituye a N28 (cambio del aminoácido N28Q, que genera el identificador de secuencia n.°: 20). En un aspecto, la HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que S sustituye a N28 (cambio del aminoácido N28S, que genera el identificador de secuencia n.°: 21). En un aspecto, la

HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que D sustituye a N28 (cambio del aminoácido N28D, que genera el identificador de secuencia n.°: 22). En un aspecto, la HVR-L1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 9 en la que I sustituye a N28 (cambio del aminoácido N28I, que genera el identificador de secuencia n.°: 23). En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una HVR-L1 que comprende la secuencia de aminoácidos de cualquiera de los identificadores de secuencia n.° 9, 10, 19, 20, 21, 22, 23. En un aspecto, la HVR-L1 es cualquiera de los identificadores de secuencia n.° 9, 10, 19, 20, 21, 22 ó 23 y A sustituye al aminoácido en la posición N30 (asparagina en posición 30) (cambio del aminoácido N30A). En un aspecto, la HVR-L1 es cualquiera de los identificadores de secuencia n.° 9, 10, 19, 20, 21, 22 ó 23 y Q sustituye al aminoácido en la posición N30 (asparagina en posición 30) (cambio del aminoácido N30Q).

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende (a) una HVR-H3 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 6 y (b) una HVR-L3 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 14. En ciertas formas de realización, el anticuerpo CD22 además comprende (a) una HVR-H1 que comprende un identificador de secuencia n.°: 2 y una HVR-H2 que comprende un identificador de secuencia n.°: 4.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende (a) una HVR-H3 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 6 y (b) una HVR-L2 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 12. En ciertas formas de realización, el anticuerpo CD22 además comprende (a) una HVR-H1 que comprende un identificador de secuencia n.°: 2 y una HVR-H2 que comprende un identificador de secuencia n.°: 4.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende (a) una HVR-H3 que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 6 y (b) una HVR-L1 que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 9, 10, 19, 20, 21, 22 y 23. En ciertas formas de realización, el anticuerpo CD22 además comprende (a) una HVR-H1 que comprende un identificador de secuencia n.°: 2 y una HVR-H2 que comprende un identificador de secuencia n.°: 4. En ciertas formas de realización, la secuencia de aminoácidos del identificador de secuencia n.°: 9, 10, 19, 20, 21, 22 ó 23 comprende un cambio del aminoácido N30Q o N30A. En ciertas formas de realización, el anticuerpo CD22 además comprende la HVR-L2 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 12. En ciertas formas de realización, el anticuerpo CD22 además comprende la HVR-L3 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 14.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende (a) una HVR-H1 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 2; (b) una HVR-H2 que comprende la secuencia de aminoácidos del identificador de secuencia n°. 4; (c) una HVR-H3 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 6; (d) una HVR-L1 que comprende la secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 9, 10, 19, 20, 21, 22, 23; (e) una HVR-L2 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 12 y una HVR-L3 que comprende la secuencia de aminoácidos del identificador de secuencia n.°: 14. En ciertas formas de realización, la invención además proporciona la secuencia de aminoácidos del identificador de secuencia n.°: 9, 10, 19, 20, 21, 22 ó 23 seleccionada cuando HVR-L1 se ve modificada por un cambio del aminoácido N30A o N30Q.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena pesada que comprende un identificador de secuencia n.°: 16 (véase la Figura 2A, h10F4v1). En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena ligera que comprende un identificador de secuencia n.°: 17 (véase la Figura 2B, h10F4v1). En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena ligera que comprende un identificador de secuencia n.°: 18 (véase la Figura 2B, h10F4v3).

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una cadena pesada que comprende un identificador de secuencia n.°: 34 (véase la Figura 2A, m10F4). En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende una cadena ligera que comprende un identificador de secuencia n.°: 35 (véase la Figura 2B, m10F4).

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende 1, 2, 3, 4, 5 ó 6 de las secuencias de la HVR del anticuerpo 10F4.4.1 producido por el hibridoma depositado con la ATCC y con entrada número PTA-7621.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende 1, 2, 3, 4, 5 ó 6 de las secuencias de la HVR del anticuerpo 5E8.1.8 producido por el hibridoma depositado con la ATCC y con entrada número PTA-7620.

Un anticuerpo anti-CD22 puede comprender cualquier secuencia de dominio variable de la estructura adecuada, siempre que el anticuerpo conserve la capacidad de unirse a CD22. Por ejemplo, en ciertas formas de realización, los anticuerpos anti-CD22 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 71, 73 ó 78. En una forma de realización de estos anticuerpos, la posición 71 corresponde a A, la posición 73 corresponde a T o la posición 78 corresponde a A. 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.°: 1, 3, 5, 7 (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.° 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 I 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.°: 8, 1, 13, 15 (FR-L1, FR-L2, FR-L3, FR-L4, respectivamente).

En una forma de realización, un anticuerpo anti-CD22 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.°: 1, 3, 5 y 7 respectivamente; la HVR-H1 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 2; la HVR-H2 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 4 y la HVR-H3 comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 6. En una forma de realización, un anticuerpo anti-CD22 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 n°. 8, 11, 13 y 15 respectivamente; la HVR-L1 comprende la secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 9, 10, 19, 20, 21, 22 y 23, en las que cualquiera de los identificadores de secuencia n.° 9-10 ó 19-23 puede comprender un cambio del aminoácido N30A o N30Q; la HVR-L2 comprende la secuencia de aminoácidos del identificador de secuencia n.°: 12 y la HVR-L3 comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 14. En una forma de realización de estos anticuerpos, el dominio variable de cadena pesada comprende un identificador de secuencia n.°: 16 y el dominio variable de cadena ligera comprende el identificador de secuencia n.°: 17 ó 18.

En ciertas formas de realización, la invención proporciona un anticuerpo anti-CD22 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.°: 16. 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 CD22. 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.°: 16. 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-CD22 comprende un dominio variable de cadena pesada que contiene una secuencia de aminoácidos del identificador de secuencia n.°: 16.

En ciertas formas de realización, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena pesada, tal como se describe a continuación.

1 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 113 (Identificador de secuencia n.°: 16) (Los residuos de la HVR aparecen subrayados).

En ciertas formas de realización, las secuencias de la HVR y FR de cadena pesada comprenden los siguientes elementos:

HVR-H1 (Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn, identificador de secuencia n.°: 2)

HVR-H2 (Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys Phe Lys Gly, identificador de secuencia n.°: 4)

HVR-H3 (Asp Gly Ser Ser Trp Asp Try Tyr Phe Asp Tyr, identificador de secuencia n.°: 6)

FR-H1 (Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser, identificador de secuencia n.°: 1)

FR-H2 (Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val, identificador de secuencia n.°: 3)

FR-H3 (Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg, identificador de secuencia n.°: 5)

FR-H4 (Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser, identificador de secuencia n.°: 7)

En ciertas formas de realización, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena ligera, tal como se describe a continuación.

1 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 108

(Identificador de secuencia n.°: 17) (Los residuos de la HVR aparecen subrayados y la posición N28 aparece en negrita.)

o

1 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg 108 (Identificador de secuencia n.°: 18) (Los residuos de la HVR aparecen subrayados y la posición N28V aparece en negrita.)

En ciertas formas de realización, las secuencias de la HVR de cadena ligera comprenden los siguientes elementos:

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 9)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 10)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ala Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 19)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Gln Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 20)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 21)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Asp Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 22)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Asn Thr Phe Leu Glu, identificador de secuencia n.°: 23)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Ala Thr Phe Leu Glu, identificador de secuencia n.°: 32)

HVR-L1 (Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Gln Thr Phe Leu Glu, identificador de secuencia n.°: 33)

HVR-L2 (Lys Val Ser Asn Arg Phe Ser, identificador de secuencia n.°:12)

HVR-L3 (Phe Gln Gly Ser Gln Phe Pro Tyr Thr, identificador de secuencia n.°: 14).

En ciertas formas de realización, las secuencias de la FR de cadena ligera comprenden los siguientes elementos:

FR-L1 (Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys, identificador de secuencia n.°: 8);

FR-L2 (Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr, identificador de secuencia n.°: 11);

FR-L3 (Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys, identificador de secuencia n.°: 13)

FR-L4 (Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg, identificador de secuencia n.°: 15).

En un aspecto, la invención proporciona un anticuerpo anti-CD22 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 seleccionada del identificador de secuencia n.°: 17 ó 18. 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 CD22. 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.°: 17 ó 18. 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-CD22 comprende un dominio variable de cadena ligera que contiene una secuencia de aminoácidos seleccionada a partir del identificador de secuencia n.°: 17 ó 18.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 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.°: 16 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 seleccionada del identificador de secuencia n.°: 17 ó 18. 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 CD22. En ciertas formas de realización, un total de entre 1 y 10 aminoácidos se han sustituido, insertado o eliminado en la secuencia de referencia. 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-CD22 comprende un dominio variable de cadena pesada que comprende una secuencia de aminoácidos del identificador de secuencia n.°: 16 y un dominio variable de cadena ligera que comprende una secuencia de aminoácidos seleccionada del identificador de secuencia n.°: 18.

En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende (a) una, dos o tres HVR de VH seleccionadas de las que se muestran en la Figura 2A o (b) una, dos o tres HVR de VL seleccionadas de las que se muestran en la Figura 2B. En un aspecto, la invención proporciona un anticuerpo anti-CD22 que comprende un dominio variable de cadena pesada seleccionado entre los que se muestran en la Figura 2A y un dominio variable de cadena ligera seleccionado entre los que se muestran en la Figura 2B.

En un aspecto, el anticuerpo anti-CD22 de la invención comprende 1, 2, 3, 4, 5 ó 6 de las regiones hipervariables del anticuerpo 5E8.1.8 producido por el hibridoma depositado con la ATCC y con entrada número PTA-7620.

2. *Fragmentos de anticuerpos*

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.

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 huésped 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 huésped 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*

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. N° 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.

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).

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

Los anticuerpos humanos anti-CD22 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-CD22 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).

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).

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

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 CD22 y la otra, a cualquier otro antígeno. En ciertas formas de realización, los anticuerpos biespecíficos se pueden unir a dos epítopos diferentes de CD22. Los anticuerpos biespecíficos también se pueden utilizar para localizar agentes citotóxicos de las células que expresan el CD22. Estos anticuerpos poseen un brazo de unión a CD22 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 $F(ab')_2$).

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).

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 huésped 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.

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).

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.

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

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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.

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.

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.

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).

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

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

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

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.

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.

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.

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.

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.

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.° 2003/0157108 (Presta, L.). Véase también patente de EE.UU. N.° 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.° 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.

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)).

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 5834597).

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)	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; Norleucina	Leu
Leu (L)	Norleucina; 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; Tir	Tir
Pro (P)	Ala	Ala
Ser (S)	Tr	Tr
Tr (T)	Val; Ser	Ser
Trp (W)	Tir; Phe	Tir
Tir (Y)	Trp; Phe; Tr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucina	Leu

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)

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: Cys, 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.

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).

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.

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.

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.

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.° 5.648.260; la patente de EE. UU. N.° 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.° 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.° 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).

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.° 5.731.168.

9. Derivados del anticuerpo

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 polipropileno / 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.

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

Pueden conseguirse los anticuerpos monoclonales anti-CD22 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).

En el método del hibridoma, un ratón u otro animal huésped 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-CD22 se producen generalmente en animales mediante inyecciones subcutáneas (sc) o intraperitonales (ip) de CD22 y un adyuvante. El CD22 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 CD22 se puede producir de forma recombinante. En una forma de realización, se inmuniza a los animales con un derivado de CD22 que contiene una porción extracelular de CD22 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 CD22-IgG1. En una forma de realización, se inmuniza a animales con derivados inmunogénicos de CD22 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-CD22. Los animales reciben dosis de refuerzo hasta obtener los niveles adecuados de titulación.

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)).

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.

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)).

El medio de cultivo en el cual las células de hibridoma crecen se analiza para la producción de anticuerpos monoclonales que se unan a CD22. 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).

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*

Los anticuerpos anti-CD22 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.

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-CD22 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-CD22 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.

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".

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).

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 huésped 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).

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-CD22, el sujeto se inmuniza con CD22 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-CD22 humano se obtiene al generar una respuesta a un anticuerpo anti-CD22 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 CD22 da lugar a células B productoras de anticuerpos humanos anti-CD22. La generación de ratones transgénicos productores de anticuerpos humanos se describe más adelante.

El enriquecimiento adicional de poblaciones celulares reactivas a anti-CD22 se puede obtener mediante un procedimiento de detección adecuado para aislar linfocitos B que expresen un anticuerpo unido a membrana específico de CD22, por ejemplo, mediante separación celular con cromatografía de afinidad a CD22 o adsorción de células a CD22 marcada con flurocromo seguida de separación de células activadas por fluorescencia (FACS).

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 CD22 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.

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).

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).

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).

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).

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.

La detección de las bibliotecas se puede lograr mediante diversas técnicas conocidas en la materia. Por ejemplo, se puede utilizar CD22 para revestir los pocillos de placas de adsorción, expresado en huéspedes 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.

Las muestras de bibliotecas de fagos están en contacto con el CD22 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 CD22, 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.

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).

Es posible seleccionar entre anticuerpos de fagos de distintas afinidades, incluso con afinidades que difirieren ligeramente, para CD22. 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 CD22 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 CD22 biotinilado, pero con el CD22 biotinilado a una concentración de molaridad menor de la constante de afinidad molar diana para CD22. 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.

Los clones anti-CD22 se pueden seleccionar en función de la actividad. En ciertas formas de realización, la invención proporciona anticuerpos anti-CD22 que se unen a células vivas que expresan naturalmente el CD22. En una forma de realización, la invención proporciona anticuerpos anti-CD22 que bloquean la unión entre un ligando de CD22 y CD22, pero no bloquean la unión entre un ligando de CD22 y una segunda proteína. Los clones de Fv que corresponden a estos anticuerpos anti-CD22 se pueden seleccionar (1) aislando clones anti-CD22 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 huésped bacteriano apropiado; (2) seleccionando CD22 y una segunda proteína contra la que se desea una actividad bloqueante y no bloqueante, respectivamente; (3) adsorbiendo los clones de fagos anti-CD22 a CD22 inmovilizado; (4) usando una mayor cantidad de la segunda proteína para eluir los clones no deseados que reconocen los determinantes de unión a CD22 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.

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).

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.

El ADN que codifica un anticuerpo anti-CD22 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 huésped y métodos recombinantes

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 huésped celular que se utilice. Por lo general, las células huésped 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 huésped eucarióticas:

Construcción de vectores

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 huéspedes 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 huésped 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 huésped 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.

En general, los vectores plásmidos que contienen secuencias de control y replicón que se derivan de especies compatibles con la célula huésped se usan en conexión con estos huéspedes. 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.

Además, los vectores fágicos que contienen secuencias de control y replicón que son compatibles con los microorganismos huésped se pueden usar como vectores de transformación en relación con estos huéspedes. Por ejemplo, un bacteriófago como λGEM.TM.-11 se puede utilizar para marcar un vector recombinante que se pueda utilizar para transformar células huésped susceptibles como *E. coli* LE392.

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.

Se conoce un gran número de promotores reconocidos por diversas células huésped 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.

Los promotores aptos para su uso con huéspedes 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.

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 huésped reconoce y procesa (es decir, escindida por una peptidasa de señal). Para las células huésped 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.

En otro aspecto, la producción de las inmunoglobulinas según la invención puede tener lugar en el citoplasma de la célula huésped 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 huéspedes (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)).

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.

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.

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.

Las células huésped 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 huéspedes 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 huésped cuando plásmidos bien conocidos como pBR322, pBR325, pACYC177 o pKN410 se utilizan para suministrar al replicón. Normalmente, en caso de que la célula huésped deba secretar cantidades mínimas de enzimas proteolíticas puede resultar conveniente incorporar al cultivo celular inhibidores adicionales de la proteasa.

Producción de anticuerpos

Las células huésped 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.

Transformación significa introducir ADN en el huésped procariótico de forma que el ADN sea replicable, ya sea como elemento extracromosómico o como integrante cromosómico. Según la célula huésped 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.

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 huésped 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.

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.

Las células huésped 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 huésped. En ciertas formas de realización, el pH para *E. coli* puede oscilar entre 6,8 y 7,4 aproximadamente o 7,0 aproximadamente.

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 huésped 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.

En una forma de realización, los polipéptidos expresados de la presente invención se secretan en el periplasma de las células huésped 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.

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.

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.

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 cys,trans-isomerasa con actividad chaperona) para co-transformar las células huésped procarióticas. Se ha demostrado que las proteínas chaperonas facilitan la solubilidad y el plegamiento adecuado de las proteínas heterólogas producidas en células bacterianas huésped. 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.

Pueden utilizarse ciertas cepas huésped 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 huésped 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).

En una forma de realización, en el sistema de expresión de la invención se utilizan como células huésped cepas de *E. coli* con deficiencia de enzimas proteolíticas transformadas con plásmidos que sobreexpresan una o más proteínas chaperonas.

Purificación de anticuerpos

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.

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.

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 huésped eucarióticas:

Un vector para utilizar en una célula huésped 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

Un vector para utilizar en una célula huésped 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 huésped 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

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

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.

Un ejemplo de un esquema de selección utiliza un fármaco para detener el crecimiento de una célula huésped. 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.

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.

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 huésped 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).

Alternativamente, las células huésped (en especial los huéspedes 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

Los vectores de expresión y clonación suelen contener un promotor que es reconocido por el organismo huésped 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.

La trascripción a partir de los vectores en células huésped 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 huésped.

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 huéspedes 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

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

Los vectores de expresión utilizados en células huésped 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 huésped

Entre las células huésped apropiadas para clonación o expresión del ADN en los vectores de la presente memoria descriptiva figuran las células eucariotas más altas, incluidas las células huésped 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).

Las células huésped 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 huésped

Las células huésped 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 huésped. 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 huésped. 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 huésped seleccionada para la expresión y serán evidentes para los expertos.

Purificación del anticuerpo

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 huésped 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.

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.

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).

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

La invención también proporciona inmunoconjugados (también denominados "conjugados de anticuerpos con fármacos" o "ADC"), que comprenden cualquiera de los anticuerpos anti-CD22 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).

En ciertas formas de realización, un inmunoconjugado comprende un anticuerpo anti-CD22 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.

En ciertas formas de realización, un inmunoconjugado comprende un anticuerpo anti-CD22 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 – Conjugados de anticuerpos con fármacos

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-CD22 se conjuga (es decir, se une covalentemente) con una o más fracciones de fármaco (D) a través de un enlazador opcional (L).

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

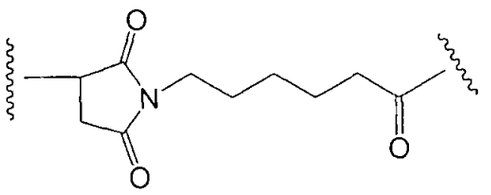
Por consiguiente, el anticuerpo anti-CD22 se puede conjugar con el fármaco ya sea directamente o mediante un enlazador. En la Fórmula I, p es el promedio de fracciones de fármaco por anticuerpo, que puede oscilar, por ejemplo, entre aproximadamente 1 y aproximadamente 20, fracciones de fármaco por anticuerpo y, en ciertas formas de realización, entre 1 y aproximadamente 8 fracciones de fármaco por anticuerpo.

Enlazadores ejemplares

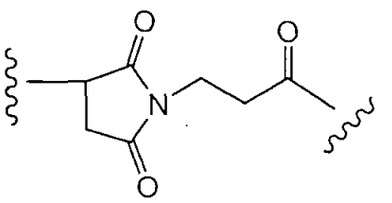
En la presente memoria descriptiva se detallan enlazadores y fracciones de fármacos ejemplares. Un enlazador puede comprender uno o más componentes enlazadores. Los componentes de enlace ejemplares incluyen: 6-maleimidocaproilo ("MC"), maleimidopropanoilo ("MP"), valina-citrulina ("val-cit" o "vc"), alanina-fenilalanina ("ala-phe"), p-aminobensiloxicarbonil ("PAB"), N-Succinimidil 4-(2-piridiltio) pentanoato ("SPP"), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato ("SMCC"), y N-Succinimidil (4-yodo-acetil) aminobenzoato ("SIAB"). Diversos componentes enlazadores son conocidos en la materia y algunos se describen más adelante.

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.° 5.208.020).

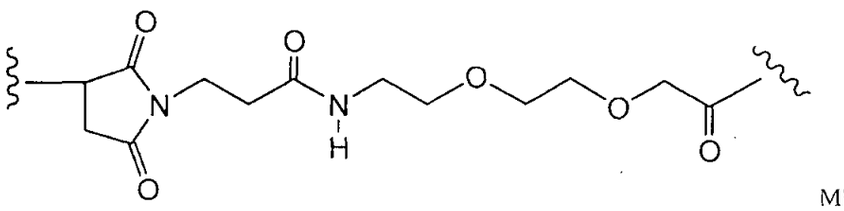
En ciertas formas de realización, un componente enlazador puede comprender una "unidad de extensión" que une un anticuerpo con otro componente enlazador o con una fracción de fármaco. A continuación se muestran unidades de extensión ejemplares (en las que la línea ondulada indica sitios de enlace covalente a un anticuerpo):



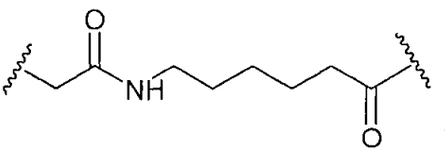
MC



MP



MPEG

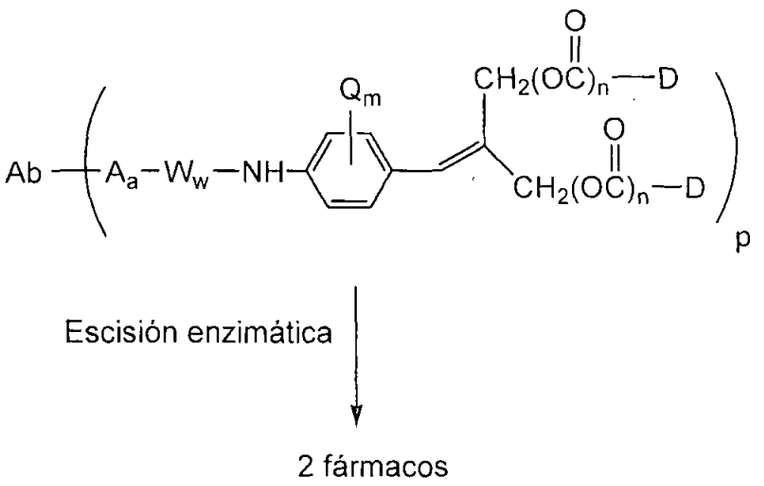


En ciertas formas de realización, un componente enlazador puede comprender una unidad de aminoácidos. En una de estas formas de realización, la unidad de aminoácidos permite la escisión del enlazador por una proteasa, lo que facilita la liberación del fármaco desde el inmunoconjugado tras exposición a proteasas intracelulares, como enzimas lisosomales. Véase, por ejemplo, Doronina y col., (2003) *Nat. Biotechnol.* 21:778-784. Entre las unidades de aminoácidos ejemplares se encuentra un dipéptido, un tripéptido, un tetrapéptido y un pentapéptido. Los dipéptidos ejemplares incluyen: valina-citrulina (vc o val-cit), alanina-fenilalanina (af o ala-phe); fenilalanina-lisina (fk o phe-lys); o N-metil-valina-citrulina (Me-val-cit). Los tripéptidos ejemplares incluyen: glicina-valina-citrulina (gly-val-cit) y glicina-glicina-glicina (gly-gly-gly). Una unidad de aminoácidos puede comprender residuos de aminoácidos que ocurren de forma natural, así como aminoácidos menores y análogos de aminoácidos que no ocurren de forma natural, como la citrulina. Las unidades de aminoácidos pueden designarse y optimizarse en su selectividad para la escisión mediante una enzima específica, por ejemplo, una proteasa asociada a un tumor, catepsina B, C y D, o una proteasa plasmina.

En algunas formas de realización, un componente enlazador puede comprender una unidad "espaciadora" que une el anticuerpo a una fracción de fármaco, bien directamente bien mediante una unidad de extensión y/o una unidad de aminoácidos. Una unidad espaciadora puede ser "autoinmoladota" o "no autoinmoladota". Una unidad espaciadora "no autoinmoladota" es aquella en la que la totalidad o parte de la unidad espaciadora permanece unida a la fracción del fármaco tras escisión enzimática (por ejemplo, proteolítica) del ADC. Entre los ejemplos de unidades espaciadoras no autoinmoladoras están una unidad espaciadora de glicina y una unidad espaciadora de glicina-glicina. También se consideran otras combinaciones de espaciadores peptídicos susceptibles de escisión enzimática específica para una secuencia. Por ejemplo, la escisión enzimática de un ADC que contenga una unidad espaciadora de glicina-glicina mediante una proteasa asociada a una célula tumoral daría lugar a la liberación de la fracción de fármaco de glicina-glicina desde el resto del ADC. En una de estas formas de realización, la fracción de fármaco de glicina-glicina está entonces sujeta a una fase independiente de hidrólisis, lo que permite la escisión de la unidad espaciadora de glicina-glicina de la fracción del fármaco.

Una unidad espaciadora "autoinmoladora" permite la liberación de la fracción del fármaco sin una fase de hidrólisis independiente. En determinadas formas de realización, una unidad espaciadora de un enlazador comprende una unidad p-aminobenzilo. En una de estas formas de realización, un alcohol p-aminobenzilo se une a una unidad de aminoácidos mediante una unión amida, y un carbamato, metilcarbamato o carbonato se realiza entre el alcohol de bencilo y un agente citotóxico. Véase, por ejemplo, Hamann y col., (2005) *Expert Opin. Ther. Patents* (2005) 15:1087-1103. En una forma de realización, la unidad espaciadora es p-aminobenciloxicarbonilo (PAB). En ciertas formas de realización, la porción de fenileno de una unidad de benzilo p-amino se sustituye por Qm, 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. Otros ejemplos de unidades espaciadoras autoinmoladoras incluyen, entre otros, compuestos aromáticos que son electrónicamente similares a alcohol p-aminobencilo (véase, por ejemplo, patente de EE.UU. N.° 2005/0256030 A1), como derivados de 2-aminoimidazol-5-metanol (Hay y col., (1999) *Bioorg. Med. Chem. Lett.* 9:2237) y orto- o para-aminobencilacetales. Se pueden utilizar espaciadores que se sometan a ciclización tras hidrólisis de la unión amida, como amidas a ácido 4-aminobutírico sustituido y no sustituido (Rodrigues y col., *Chemistry Biology*, 1995, 2, 223); sistemas en anillo biciclo[2.2.1] y biciclo[2.2.2] sustituidos adecuadamente (Storm y col., *J. Amer. Chem. Soc.*, 1972, 94, 5815); y amidas de ácido 2-aminofenilpropiónico (Amsberry y col., *J. Org. Chem.*, 1990, 55, 5867). La eliminación de fármacos con contenido en aminas que se sustituyen en la posición a de la glicina (Kingsbury y col., *J. Med. Chem.*, 1984, 27, 1447) también constituye un ejemplo de espaciadores autoinmoladores útiles en ADC.

En una forma de realización, una unidad espaciadora es una unidad ramificada bis(hidroximetil)estireno (BHMS) como se muestra a continuación, que se puede utilizar para incorporar y liberar múltiples fármacos.



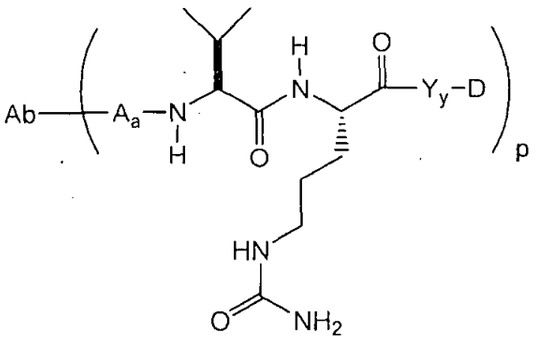
donde Q es -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -halógeno, -nitro o -ciano; m es un número entero que oscila entre 0 y 4; n es 0 ó 1; y p oscila entre 1 y aproximadamente 20.

Un enlazador puede comprender uno o más componentes enlazadores anteriores. En determinadas formas de realización, un enlazador es como se muestra entre corchetes en la siguiente Fórmula II de ADC.

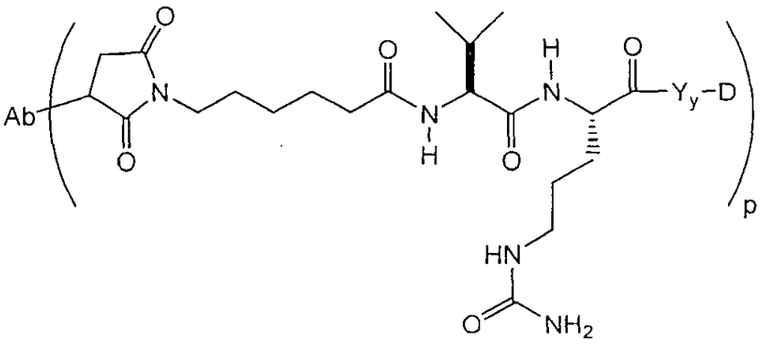
Ab-([Aa-Ww-Yy]–D)$_p$ Fórmula II

donde A es una unidad de extensión y a es un número entero comprendido entre 0 y 1; W es una unidad de aminoácidos y w es un número entero comprendido entre 0 y 12; Y es una unidad espaciadora e y es 0, 1 ó 2; y Ab, D y p se definen como se ha hecho anteriormente para la Fórmula I. Formas de realización ejemplares de estos enlazadores se describen en la patente de EE.UU. N.º 20050238649 A1, que se incorpora expresamente en la presente memoria descriptiva a modo de referencia.

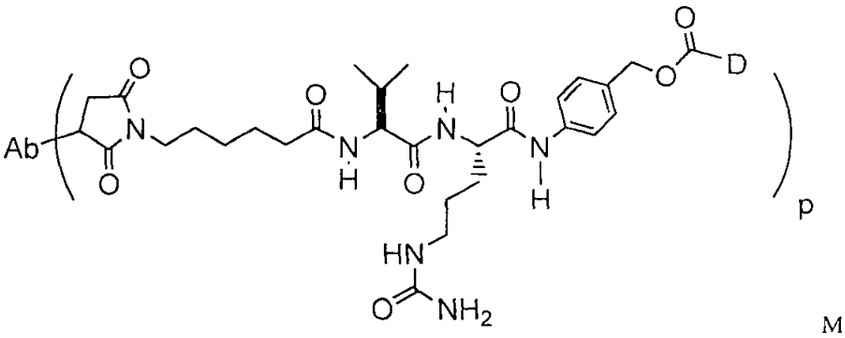
Los componentes enlazadores ejemplares y las combinaciones de los mismos se muestran a continuación en el contexto de los ADC de la Fórmula II:



Val-Cit o VC



MC-val-cit



MC-val-cit-PAB

Los componentes enlazadores, incluyendo las unidades de extensión, espaciadora y de aminoácidos, se pueden sintetizar mediante métodos conocidos en el sector, como los descritos en la patente de EE.UU. N.º 2005-0238649 A1.

Fracciones de fármacos ejemplares

Maitansina y maitansinoides

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 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 maitansinol (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.

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 enlazadores no disulfuro con anticuerpos, (iii) poseen estabilidad en plasma, y (iv) resultan eficaces frente a distintas líneas celulares tumorales.

Los compuestos de maitansina aptos para uso como fracciones 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). El maitansinol y los análogos del maitansinol también se pueden preparar de forma sintética mediante métodos conocidos.

A continuación se citan algunas formas de realización ejemplares de fracciones de fármacos maitansinoides: DM1; DM3; y DM4, se detallan en la presente memoria descriptiva.

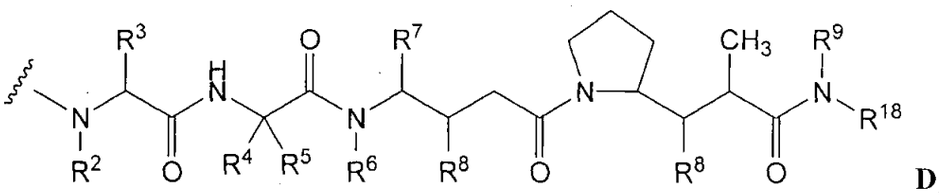
Auristatinas y dolastatinas

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. N.º 5635483; 5780588). 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 tiene actividad antineoplásica (patente de EE.UU. N.º 5663149) 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).

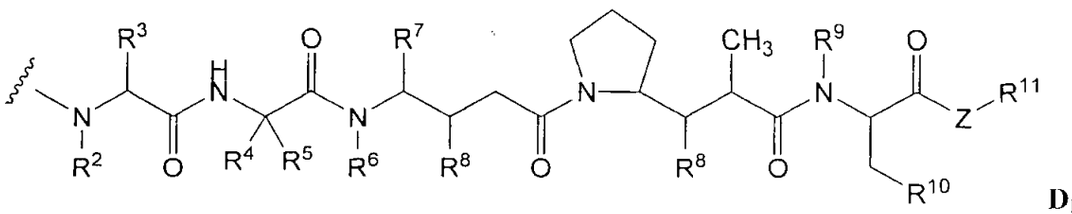
Entre las formas de realización ejemplares de auristatina se incluyen las fracciones DE y DF de fármacos de monometilauristatina enlazados al extremo N-terminal, descritas en Senter y col., *Proceedings*

of the American Association for Cancer Research, Volume 45, Abstract Number 623, presentada el 28 de marzo de 2004, cuyo contenido se incorpora expresamente en su totalidad a modo de referencia.

Una fracción peptídica de un fármaco se puede seleccionar entre las Fórmulas D_E and D_F siguientes:



$\mathbf{D_E}$



$\mathbf{D_F}$

donde la línea ondulada de D_E y D_F indica el sitio de enlace covalente a un anticuerpo o a un componente enlazador a anticuerpo, e independientemente en cada ubicación:

R^2 se selecciona entre H y C_1-C_8 alquilo;

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, C_1-C_8 alquilo y C_3-C_8 carbociclo, 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, C_1-C_{20} alquilo, arilo, C_3-C_8 heterociclo, $-(R^{13}O)_m-R^{14}$, o $-(R^{13}O)_m-CH(R^{15})_2$;

m es un número entero comprendido entre 1 y 1000;

R^{13} es C_2-C_8 alquilo;

R^{14} es H o C_1-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

m es un número entero comprendido entre 0 y 6.

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.

En otra forma de realización, R^2 y R^6 son metilo y R^9 es -H.

En otra forma de realización, cada R^8 es $-OCH_3$.

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.

En una forma de realización, Z es -O- o -NH-.

En una forma de realización, R^{10} es arilo.

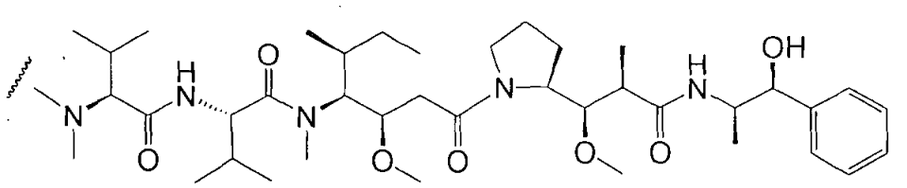
En una forma ejemplar de realización, R^{10} es -fenilo.

En una forma ejemplar de realización, cuando Z es -O-, R^{11} es –H, metilo o t-butilo.

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 $-(CH_2)_n-COOH$.

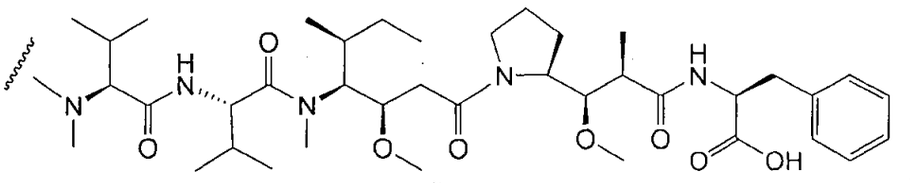
En otra forma de realización, cuando Z es -NH, R^{11} es $-CH(R^{15})_2$, donde R^{15} es $-(CH_2)_n-SO_3H$.

Una forma ejemplar de realización con auristatina de la fórmula D_E es MMAE, donde la línea ondulada indica el enlace covalente a un enlazador (L) de un conjugado de anticuerpo con fármaco:



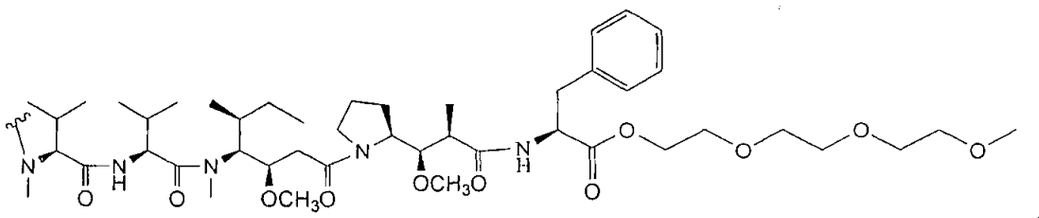
MMAE

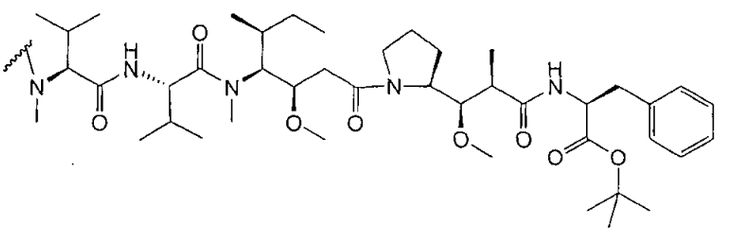
Una forma ejemplar de realización con auristatina de la fórmula D_F es MMAF, donde la línea ondulada indica el enlace covalente a un enlazador (L) de un conjugado de anticuerpo con fármaco (véase la patente de EE.UU. N.º 2005/0238649 y Doronina y col. (2006) *Bioconjugate Chem.* 17:114-124):

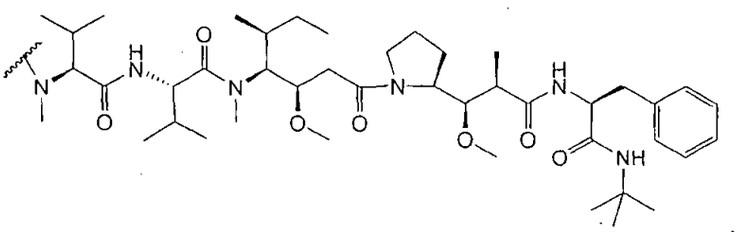


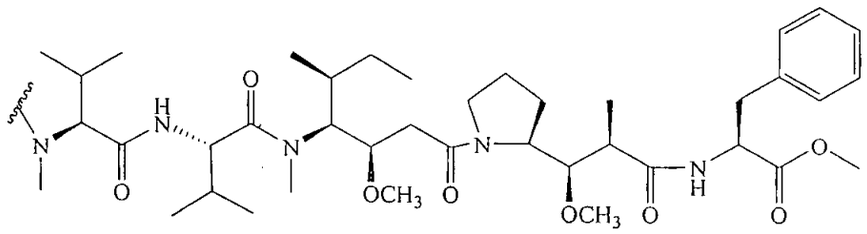
MMAF

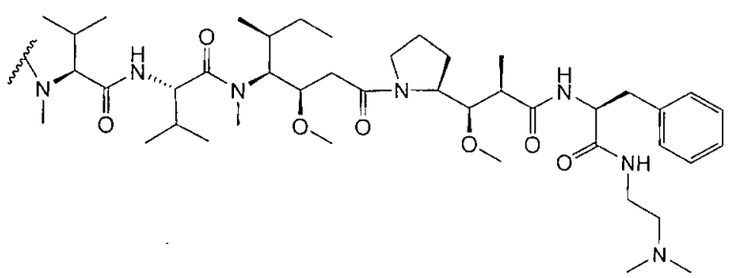
Otras fracciones de fármaco incluyen los siguientes derivados de MMAF, donde la línea ondulada indica el enlace covalente a un enlazador (L) de un conjugado de anticuerpo con fármaco:

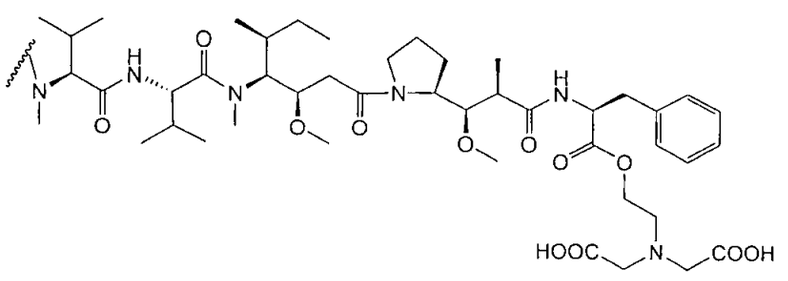


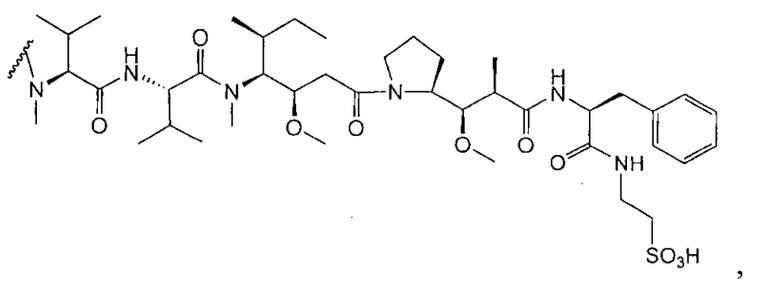


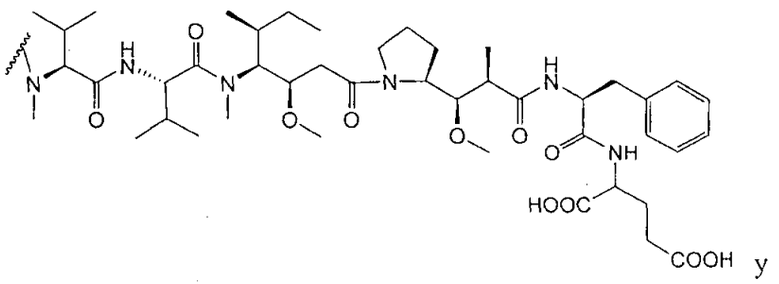




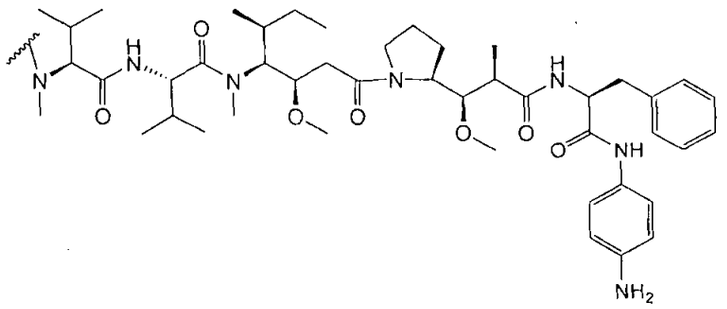






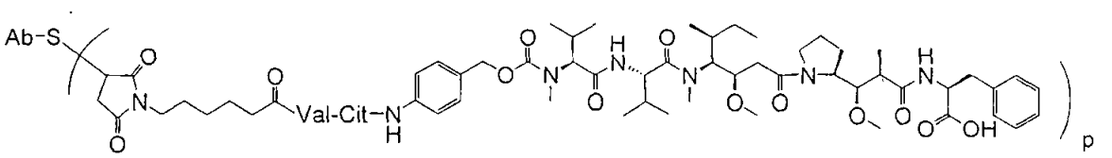


y

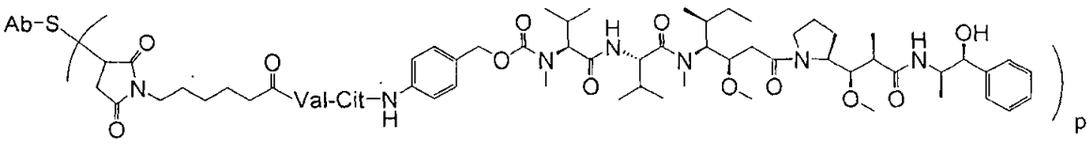


En un aspecto, los grupos hidrofílicos que incluyen, entre otros, ésteres de glicol trietileno (TEG), como se muestra anteriormente, se pueden unir a la fracción del fármaco en R^{11}. Sin unión mediante una teoría concreta, los grupos hidrofílicos ayudan a la internalización y no aglomeración de la fracción del fármaco.

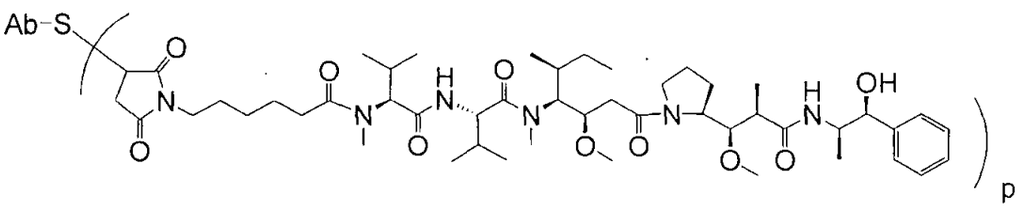
Las formas ejemplares de realización de los ADC de la Fórmula I que comprenden una auristatina/dolastatina o un derivado de la misma se describen en la patente de EE.UU. N.º 2005-0238649 A1 y en Doronina y col. (2006) *Bioconjugate Chem.* 17:114-124, que se incorpora expresamente en la presente memoria descriptiva a modo de referencia. Formas ejemplares de realización de los ADC de Fórmula I que comprenden MMAE o MMAF y diversos componentes enlazadores tienen las siguientes estructuras y abreviaturas (donde "Ab" es un anticuerpo; p está comprendido entre 1 y aproximadamente 8, "Val-Cit" es un dipéptido valina-citrulina; y "S" es un átomo de sulfuro.



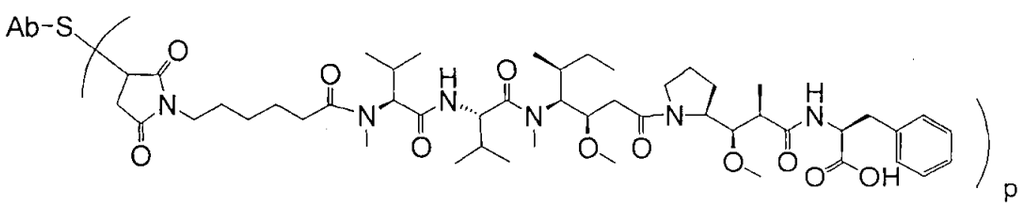
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

Las formas de realización ejemplares de los ADC de la Fórmula I que comprenden MMAF y varios componentes enlazadores 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 enlazador que no sea proteolíticamente escindible poseen una actividad similar a la de los inmunoconjugados que comprenden MMAF unido a un anticuerpo mediante un enlazador 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. Id.

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*", volumen 1, págs. 76-136, 1965, Academic Press) que es bien conocido en el campo de la química de péptidos. Las fracciones de fármacos auristatina/dolastatina pueden prepararse de acuerdo con los métodos siguientes: patente de EE.UU. N.º 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-Cancer Drug Design* 13:243-277; Pettit, G.R., y col., *Synthesis*, 1996, 719-725; Pettit y col., (1996) *J. Chem. Soc. Perkin Trans.* 15:859-863; y Doronina (2003) *Nat. Biotechnol.* 21(7):778-784.

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 la patente de EE.UU. N.º 2005-0238649 A1 y Doronina et al. (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 et al. (2003) *Nat. Biotech.* 21:778-784. Las fracciones del enlazador a fármacos MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF y MC-vc-PAB-MMAE se pueden sintetizar convenientemente 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 de EE.UU. N.º 2005/0238649 A1, y, luego conjugar con un anticuerpo de interés.

Concentración del fármaco

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 espectroscopia 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.

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 enlazador. 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 ciertas formas de realización, la concentración de fármaco para un ADC de la invención oscila entre 1 y aproximadamente 8; entre aproximadamente 2 y aproximadamente 6; entre aproximadamente 3 y aproximadamente 5; entre aproximadamente 3 y aproximadamente 4; entre aproximadamente 3,1 y aproximadamente 3,9; entre

aproximadamente 3,2 y aproximadamente 3,8; entre aproximadamente 3,2 y aproximadamente 3,7; entre aproximadamente 3,2 y aproximadamente 3,6: entre aproximadamente 3,3 y aproximadamente 3,8; entre aproximadamente 3,3 y aproximadamente 3,7. De hecho, se ha demostrado que para determinados ADC, la proporción óptima de fracciones de fármaco por anticuerpo puede ser inferior a 8 y puede estar entre 2 y aproximadamente 5. Véase la patnete de EE.UU. N.º 2005-0238649 A1 (íntegramente incorporada en la presente memoria descriptiva a modo de referencia).

En ciertas 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 enlazador a fármaco intermedio o el reactivo del enlazador, como se señala más adelante. Solamente los grupos de lisina más reactivos pueden reaccionar con un reactivo enlazador de aminos. Por lo general, los anticuerpos no contienen muchos grupos tioles de cisteína libres y reactivos que se puedan unir a una fracción de fármaco; de hecho, la mayoría de los residuos tiol de cisteína en anticuerpos están en forma de puentes disulfuro. En ciertas 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 ciertas formas de realización, un anticuerpo está sujeto a condiciones desnaturalizantes para revelar grupos nucleofílicos reactivos como lisina o cisteína.

La concentración (proporción fármaco/anticuerpo) de un ADC se puede controlar de distintas formas, por ejemplo: (i) al limitar el exceso molar del enlazador a fármaco intermedio o reactivo enlazador del anticuerpo, (ii) al limitar el tiempo o la temperatura de reacción de la conjugación y (iii) mediante condiciones de reducción parciales o limitadas para la modificación del tiol de la cisteína, (iv) al generar mediante técnicas recombinantes la secuencia de animoácido del anticuerpo de manera tal que el número y la posición de los residuos de cisteína se modifique gracias al control del número y/o posición de las uniones de enlazador-fármaco (por ejemplo, thioMab o thioFab preparado como se describe en la presente memoria descriptiva y en el documento WO2006/034488 (íntegramente incorporado en la presente memoria descriptiva a modo de referencia)).

Hay que comprender que, cuando más de un grupo nucleofílico reacciona con un enlazador a fármaco intermedio o a un reactivo enlazador 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 unidas 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 se pueden identificar en la mezcla mediante

espectroscopia de masas y separar 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, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., y col., *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). En ciertas formas de realización, un ADC homogéneo con un único valor de concentración se puede aislar de la mezcla de la conjugación mediante electroforesis o cromatografía.

Algunos métodos de preparación de inmunoconjugados

Un 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 agente enlazador bivalente, para formar un 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 enlazador bivalente, para formar D-L, mediante un enlace covalente, seguido de la reacción con un grupo nucleofílico del anticuerpo. Los métodos ejemplares de preparación de un ADC de la Fórmula I por medio de la última vía se describen en la patente de EE.UU. N.º 20050238649 A1, que se incorpora expresamente a la presente memoria descriptiva a modo de referencia.

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. Se puede hacer que los anticuerpos sean reactivos para la conjugación con reactivos del enlazador 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. De manera alternativa, los grupos sulfidrilos adicionales pueden introducirse en los anticuerpos a través de la modificación de los residuos de lisina, 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 introducirse en un anticuerpo mediante la introducción de uno, dos, tres, cuatro o mas residuos de cisteína (por ejemplo, preparando variantes de anticuerpos que comprendan uno o más residuos de aminoácidos de cisteínas no nativas).

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 enlazador. Entre los grupos nucleofílicos útiles en un reactivo enlazador están 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 enlazador. En otra forma de realización, los 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 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 y Stroh, (1992) *Bioconjugate Chem.* 3:138-146; patente de EE.UU. N.º 5362852). Este aldehído se puede hacer que reaccione con un fragmento de fármaco o un nucleófilo enlazador.

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.

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).

Los inmunoconjugados que comprenden un anticuerpo y un agente citotóxico se pueden también 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, glutaráldehídos), 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.

Alternativamente, se puede realizar una fusión de proteínas que contengan un anticuerpo y un agente citotóxico, por ejemplo, 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 uno a otro o en forma separada por una región que codifica un péptido de enlace, el cual no destruye las propiedades deseadas del conjugado.

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 ejemplo, la avidina) que se conjuga con un agente citotóxico (por ejemplo, un radionucleótido).

2. *Inmunoconjugados ejemplares – Conjugados de anticuerpo-tio con fármacos*

Preparación de anticuerpos anti-CD22 diseñados con cisteína

El ADN que codifica una variante de la secuencia de aminoácidos de los anticuerpos CD22 diseñados con cisteína y anticuerpos anti-CD22 parentales de la invención se prepara mediante diversos métodos, entre los cuáles se encuentra el aislamiento de una fuente natural (en el caso de variantes de la secuencia de los animoácidos), preparación por mutagénesis dirigida al lugar (o mediada por oliogonucleótido) (Carter (1985) y col., *Acids Res.* 13:4431-4443; Ho y col., (1989) *Gene* (Amst.) 77:51-59; Kunkel y col., (1987) *Proc. Natl. Acad. Sci. USA* 82:488;

Liu y col., (1998) *J. Biol. Chem.* 273:20252-20260), *PCR mutagénesis* (Higuchi, (1990) en PCR Protocols, págs. 177-183, Academic Press; Ito y col., (1991) *Gene* 102:67-70; Bernhard y col., (1994) *Bioconjugate Chem.* 5:126-132; y Vallette y col., (1989) *Nuc. Acids Res.* 17:723-733), y mutagénesis por inserción de un casete (Wells y col., (1985) *Gene* 34:315-323) de un ADN preparado anteriormente que codifica el polipéptido. Los protocolos de mutagénesis, kits y reactivos se encuentran comercialmente disponibles, por ejemplo, Kit de mutagénesis dirigido a múltiples sitios QuikChange® (Stratagene, La Jolla, CA). Las mutaciones individuales también se generan mediante mutagénesis dirigida por oligonucleótido utilizando plásmidos de ADN de doble cadena como una plantilla mediante mutagénesis basada en PCR (Sambrook y Russel, (2001) *Molecular Cloning: A Laboratory Manual*, 3ª edición; Zoller y col., (1983) *Methods Enzymol.* 100:468-500; Zoller, M.J. y Smith, M. (1982) *Nucl. Acids Res.* 10:6487-6500). También se pueden construir variantes de anticuerpos recombinantes mediante la manipulación de un fragmento de restricción o mediante PCR-extensión solapada con oligonucleótidos sintéticos. Los cebadores mutagénicos codifican la(s) sustitución(es) de codones de cisteína. Se pueden utilizar técnicas de mutagénesis estándar para generar ADN que codifique dichos anticuerpos diseñados con cisteína mutante (Sambrook y col., *Molecular Cloning*, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; y Ausubel y col., *Current Protocols in Molecular Biology*, Greene Publishing y Wiley-Interscience, New York, N.Y., 1993).

Se puede utilizar la tecnología de presentación en fagos (McCafferty y col., (1990) *Nature* 348:552-553) para producir anticuerpos humanos anti-CD22 y fragmentos de anticuerpos *in vitro*, del repertorio de genes de dominios variables (V) de inmunoglobulina de donantes no inmunizados. De acuerdo con esta técnica, los genes de dominios V de los anticuerpos se clonan en pauta de lectura con un gen proteico con revestimiento mayor o menor de un bacteriófago filamentoso, como el M13 o fd, y se presentan como fragmentos de anticuerpos funcionales en la superficie de la partícula del fago. Debido a que la partícula filamentosa contiene una copia del ADN monocatenario del genoma del fago, las selecciones que se basan en las propiedades funcionales del anticuerpo también conducen a la selección del gen que codifica el anticuerpo que exhibe esas propiedades. Por lo tanto, el fago imita algunas de las propiedades de las células B (Johnson y col., (1993) *Current Opinion in Structural Biology* 3:564-571; Clackson y col., (1991) *Nature*, 352:624-628; Marks y col., (1991) *J. Mol. Biol.* 222:581-597; Griffith y col., (1993) EMBO J. 12:725-734; patente de EE.UU. N.º 5565332; patente de EE.UU. N.º 5573905; patente de EE.UU. N.º 5567610; patente de EE.UU. N.º 5229275).

Los anticuerpos anti-CD22 se pueden sintetizar químicamente utilizando metodología de síntesis de oligopéptidos conocida o se pueden preparar y purificar mediante tecnología recombinante. Se puede producir una secuencia de aminoácidos apropiada, o partes de la misma, mediante síntesis directa de péptidos utilizando técnicas de fase sólida (Steward y col., *Solid-Phase Peptide Synthesis*, (1969) W.H. Freeman Co., San Francisco, CA; Merrifield, (1963) *J. Am. Chem. Soc.*, 85:2149-2154). La síntesis de proteínas *in vitro* se puede realizar utilizando técnicas manuales o mediante automatización. La síntesis automatizada durante la fase sólida se puede lograr, por ejemplo, empleando aminoácidos protegidos con t-BOC o Fmoc y utilizando un sintetizador de péptidos de Applied Biosystems (Foster City, CA) siguiendo las instrucciones el fabricante. Diversas porciones del anticuerpo anti-CD22 o el polipéptido CD22 se pueden sintetizar químicamente de manera separada y combinar utilizando métodos químicos o enzemáticos para producir el anticuerpo anti-CD22 o el polipéptido CD22 deseados.

Se han desarrollado varias técnicas para la producción de fragmentos de anticuerpos. Tradicionalmente, estos fragmentos se obtenían mediante digestión proteolítica de anticuerpos intactos (Morimoto y col., (1992) *Journal of Biochemical and Biophysical Methods* 24:107-117; y Brennan y col., *Science,* 229:81), o se producían directamente mediante células huésped recombinantes. Los fragmentos de anticuerpos anti-CD22 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 en la presente memoria descriptiva. Como alternativa, los fragmentos Fab'-SH pueden recuperarse directamente de *E. coli* y unirse químicamente para formar fragmentos $F(ab')_2$ (Carter y col., (1992) *Bio/Technology* 10:163-167) o se pueden aislar directamente de cultivos de células huésped recombinantes. Los anticuerpos anti-CD22 pueden ser un fragmento Fv de cadena única (scFv) (documento WO 93/16185; patente de EE.UU. N.º 5571894; patente de EE.UU. N.º 5587458). El fragmento del anticuerpo anti-CD22 también puede ser un "anticuerpo lineal" (patente de EE.UU. N.º 5641870). Estos fragmentos de anticuerpos lineales pueden ser monoespecíficos o biespecíficos.

La siguiente descripción está relacionada principalmente con la producción de anticuerpos anti-CD22 mediante células de cultivo transformadas o transfectadas con un vector que contiene ácido nucleico que codifica el anticuerpo anti-CD22. Se pueden obtener anticuerpos anti-CD22 que codifican el ADN de una biblioteca de ADNc preparada con tejido que se cree que posee el ARNm del anticuerpo anti-CD22 y que lo expresa a un nivel detectable. Por consiguiente, el anticuerpo anti-CD22 humano o el ADN del polipéptido CD22 se pueden obtener fácilmente de una biblioteca de ADNc preparada a partir de tejido humano. El gen que codifica el anticuerpo anti-CD22 también se puede obtener de una biblioteca genómica o mediante los procedimientos sintéticos conocidos (por ejemplo, síntesis automatizada del ácido nucleico).

Los métodos de diseño, selección y preparación de la invención permiten la creación de anticuerpos anti-CD22 diseñados con cisteína que reaccionan con funcionalidad electrofílica. Estos métodos además permiten compuestos de conjugados de anticuerpos tales como compuestos de conjugados de anticuerpos con fármacos (ADC) con moléculas de fármaco en puntos designados, diseñados y selectivos. Los residuos reactivos de cisteína en la superficie de un anticuerpo permiten específicamente conjugar una fracción del fármaco utilizando un grupo tiol reactivo, por ejemplo, maleimida o haloacetil. La reactividad nucleofílica de la funcionalidad tiol de un residuo cys 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). Se puede calcular la cantidad de tiol libre en una proteína mediante la prueba estándar de Ellman. La inmunoglobulina M es un ejemplo de un pentámetro con enlazador disulfuro, mientras que la inmunoglobulina G es un ejemplo de una proteína con puentes disulfuros internos que unen las subunidades. En proteínas como ésta, se requiere la reducción de las uniones disulfuro con un reactivo como ditiotreitol (DTT) o selenol (Singh y col.,

(2002) *Anal. Biochem.* 304:147-156) para generar el tiol libre reactivo. Este método puede producir pérdida de la estructura terciaria del anticuerpo o especificidad de unión a antígeno.

El ensayo Pheselector (ELISA de fagos para seleccionar tioles reactivos) permite la detección de grupos de cisteína reactivos en anticuerpos en un formato de ELISA de fagos, ayudando de este modo en el diseño de los anticuerpos diseñados con cisteína (documento WO 2006/034488). El anticuerpo diseñado con cisteína se recubre en las superficies de los pocillos, seguido de incubación con partículas del fago, adición del anticuerpo secundario marcado con HRP y detección de la absorbencia. Las proteínas mutantes que reproducen en imágenes de fagos se pueden monitorear de manera rápida y sólida, y con un alto rendimiento. Las bibliotecas de anticuerpos diseñados con cisteína se pueden producir y estar sujetas a selección de unión utilizando el mismo método para diferenciar de forma apropiada los puntos reactivos de incorporación libre de Cys de bibliotecas aleatorias de proteínas-fagos de anticuerpos u otras proteínas. Esta técnica incluye proteínas mutantes que reaccionan con cisteína representadas en fagos con un reactivo de afinidad o un grupo indicador que también es reactivo del tiol.

El ensayo PHESELECTOR permite detectar grupos tiol reactivos en anticuerpos. Un ejemplo de este método es la identificación de la variante A121C. Se puede buscar eficazmente la molécula completa de Fab para identificar más variantes de ThioFab con grupos tiol reactivos. Se empleó un parámetro, área superficial accesible, para identificar y cuantificar la accesibilidad del solvente a los residuos de aminoácidos en un polipéptido. El área superficial accesible puede expresarse como la superficie ($Å^2$) que puede entrar en contacto con una molécula disolvente, por ejemplo, agua. El espacio ocupado por el agua es aproximadamente como una esfera con un radio de 1,4 Å. El software está disponible libremente o se puede adquirir mediante licencia (Secretaría CCP4, Daresbury Laboratory, Warrington, WA4 4AD, Reino Unido, Fax: (+44) 1925 603825, o por Internet: www.ccp4.Ab.uk/dist/html/INDEX.html) como el CCP4 Suite de programas de cristalografía que utiliza alogaritmos para calcular el área superficial accesible de cada aminoácido de un proteína con coordenadas conocidas derivadas de la cristalografía de rayos X ("The CCP4 Suite: Programs for Protein Crystallography" (1994) Acta. Cryst. D50:760-763). Dos módulos ejemplares de software que realizan cálculos del área superficial accesible son "AREAIMOL" y "SURFACE", basados en los alogaritmos de B. Lee y F.M. Richards (1971) *J. Mol. Biol.* 55:379-400. AREAIMOL define el área superficial accesible al solvente de un proteína como el locus del centro de una esfera de prueba (que representa una molécula de solvente) mientras que rueda sobre la superficie Van der Waals de la proteína. AREAIMOL calcula el área superficial accesible del solvente generando puntos superficiales en una esfera extendida sobre cada átomo (a una distancia del centro del átomo igual a la suma del átomo y los radios de la prueba) y eliminando los que se encuentran dentro de las esferas equivalentes asociadas a átomos adyacentes. AREAIMOL define el área superficial accesible al solvente

de átomos en un archivo PDB coordinado y resume el área a la que puede acceder el residuo, la cadena y la molécula en su totalidad. Las áreas accesibles (o diferencias del área) para átomos individuales se pueden escribir en un archivo de salida seudo-PDB. AREAIMOL asume un único radio para cada elemento y sólo reconoce un número limitado de elementos diferentes.

AREAIMOL y SURFACE brindan información sobre áreas accesibles absolutas; es decir, el número de Angtroms cuadrados (Å). El área superficial accesible se calcula mediante referencia a un estado estándar relevante para un aminoácido dentro de un polipéptido. El estado de referencia es el tripéptido Gly-X-Gly, donde X es el aminoácido de interés y el estado de referencia debe ser una conformación "extendida"; es decir, como las que se encuentran en cadenas beta. La conformación extendida maximiza la accesibilidad de X. Un área accesible calculada se divide por el área accesible en un estado de referencia de un tripéptido Gly-X-Gly y brinda el cociente, que es la accesibilidad al área superficial. El porcentaje de accesibilidad es igual a la accesibilidad al área superficial multiplicado por 100. Otro alogaritmo ejemplar para calcular la accesibilidad al área superficial se basa en el módulo SOLV del programa xsae (Broker, C., F. Hoffman-LaRoche, Basel), que calcula la accesibilidad al área superficial de un residuo de aminoácido a una esfera de agua basada en coordinadas de rayos X de un polipéptido. El área superficial accesible para cada animoácido en un anticuerpo se puede calcular utilizando información disponible sobre la estructura del cristal (Eigenbrot y col., (1993) *J Mol Biol.* 229:969-995).

Se aísla el ADN que codifica los anticuerpos diseñados con cisteína y se secuencia utilizando procedimientos convencionales (por ejemplo, usando sondas oligonucleotídicas capaces de unirse específicamente a los genes que codifican las cadenas pesadas y ligeras de anticuerpos murinos). Las células de hibridoma sirven como fuente de dicho ADN. Una vez aislado, el ADN se puede colocar en vectores de expresión, que después se transfectan en células huésped como por ejemplo células *E. coli*, células COS de monos, células de ovario de hámster chino (CHO) u otras células huésped de mamíferos, tales como células de mieloma (patente de EE.UU. N.° 5807715; patente de EE.UU. N.° 2005/0048572; patente de EE.UU. N.° 2004/0229310) que, de otro modo, no producen la proteína del anticuerpo, para obtener la síntesis de anticuerpos monoclonales en las células huésped recombinantes.

Luego del diseño y la selección, los anticuerpos diseñados con cisteína, por ejemplo, ThioFabs diseñados con los residuos de Cys altamente reactivos e independientes, se pueden reproducir por: (i) expresión en un sistema bacteriano, por ejemplo, *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 de células de mamíferos (documento WO 01/00245), por ejemplo, células de ovario de hámster chino (CHO); y (ii)

purificación utilizando técnicas comunes de purificación de proteína (Lowman y col., (1991) *J. Biol. Chem.* 266(17):10982-10988).

Los grupos tiol de cisteína diseñada reaccionan con reactivos enlazadores electrofílicos y enlazadores a fármaco intermedios para formar conjugados de anticuerpos diseñados con cisteína con fármacos y otros anticuerpos marcados diseñados con cisteína. Los residuos de cisteína de los anticuerpos diseñados con cisteína, y que se encuentran en los anticuerpos parentales, que están emparejados y forman enlaces disulfuros de la intercadena e intracadena, no tienen ningún grupo tiol reactivo (a menos que se traten con un agente reductor) y no reaccionan con reactivos enlazadores electrofílicos o enlazadores a fármaco intermedios. El residuo de cisteína recientemente diseñado puede seguir siendo independiente y reaccionar con, es decir, conjugarse a, un reactivo enlazador electrofílico o un enlazador a fármaco intermedio, tal como un fármaco-maleimida. Entre los enlazadores a fármaco intermedios se encuentran: MC-MMAE, MC-MMAF, MC-vc-PAB-MMAE y MC-vc-PAB-MMAF. Las posiciones de estructura de los residuos diseñados con cisteína de las cadenas pesadas y ligeras se enumeran de acuerdo con un sistema de numeración secuencial. Este sistema de numeración secuencial se relaciona con el sistema de numeración de Kabat (Kabat y col., (1991) *Sequences of Proteins of Immunologicla Interest*, 5th Ed, Public Health Service, National Institutes of Health, Bethesda, MD) que comienza en el extremo N-terminal, pero se diferencia del esquema de numeración de Kabat (fila inferior) mediante inserciones marcadas con a, b, c. Utilizando el sistema de numeración Kabat, la secuencia lineal real de aminoácidos puede contener menos aminoácidos o aminoácidos adicionales que correspondan a un acortamiento de una FR o CDR del domino variable o a una inserción en ellas. Los diferentes puntos de la cadena pesada diseñada con cisteína se identifican mediante los esquemas de numeración secuencial y de numeración de Kabat.

En una forma de realización, el anticuerpo anti-CD22 diseñado con cisteína se prepara mediante un proceso que comprende:

(a) sustitución de uno o más residuos de aminoácidos de un anticuerpo anti-CD22 parental por cisteína; y

(b) determinación de la reactividad del tiol del anticuerpo anti-CD22 diseñado con cisteína mediante la reacción de dicho anticuerpo con un reactivo del tiol.

El anticuerpo diseñado con cisteína puede ser más reactivo que el anticuerpo parental con el reactivo del tiol.

Los residuos de aminoácidos sin cisteína pueden situarse en las cadenas pesada o ligera, o en los dominios constante o variable. Los fragmentos de anticuerpos, por ejemplo, Fab, también pueden diseñarse con uno o más aminoácidos con cisteína que reemplacen los aminoácidos del fragmento del anticuerpo para formar fragmentos del anticuerpo diseñados con cisteína.

Otra forma de realización de la invención proporciona un método de preparación (realización) de un anticuerpo anti-CD22 diseñado con cisteína, que comprende:

(a) introducción de uno o más aminoácidos con cisteína en un anticuerpo anti-CD22 parental para generar el anticuerpo anti-CD22 diseñado con cisteína; y

(b) determinación de la reactividad del tiol del anticuerpo diseñado con cisteína con un reactivo del tiol;

donde el anticuerpo diseñado con cisteína es más reactivo que el anticuerpo parental con el reactivo del tiol.

El paso (a) del método de preparación de un anticuerpo diseñado con cisteína puede comprender:

(i) mutagenización de una secuencia de ácido nucleico que codifica el anticuerpo diseñado con cisteína;

(ii) expresión del anticuerpo diseñado con cisteína; y

(iii) aislamiento y purificación del anticuerpo diseñado con cisteína.

El paso (b) del método de preparación de un anticuerpo diseñado con cisteína puede comprender la expresión de dicho anticuerpo en una partícula de virus seleccionada de un fago o una partícula fagémida.

El paso (b) del método de preparación de un anticuerpo diseñado con cisteína también puede comprender:

(i) reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad del tiol para generar un anticuerpo diseñado con cisteína y marcado por afinidad; y

(ii) medición de la unión del anticuerpo diseñado con cisteína y marcado por afinidad a un medio de captura.

Otra forma de realización de la invención es un método de monitoreo de anticuerpos diseñados con cisteína con aminoácidos con cisteína altamente reactivos e independientes para determinar la reactividad del tiol. Este método comprende:

(a) introducción de uno o más aminoácidos con cisteína en un anticuerpo parental para generar un anticuerpo diseñado con cisteína;

(b) reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad del tiol para generar un anticuerpo diseñado con cisteína y marcado por afinidad; y

(c) medición de la unión del anticuerpo diseñado con cisteína y marcado por afinidad a un medio de captura: y

(d) determinación de la reactividad del tiol del anticuerpo diseñado con cisteína con el reactivo del tiol.

El paso (a) del método de monitoreo de un anticuerpo diseñado con cisteína puede comprender:

(i) mutagenización de una secuencia de ácido nucleico que codifica el anticuerpo diseñado con cisteína;

(ii) expresión del anticuerpo diseñado con cisteína; y

(iii) aislamiento y purificación del anticuerpo diseñado con cisteína.

El paso (b) del método de monitoreo de anticuerpos diseñados con cisteína puede comprender la expresión del anticuerpo diseñado con cisteína en una partícula de virus seleccionada de un fago o una partícula fagémida.

El paso (b) del método de monitoreo de un anticuerpo diseñado con cisteína también puede comprender:

(i) reacción del anticuerpo diseñado con cisteína con un reactivo de afinidad del tiol para generar un anticuerpo diseñado con cisteína y marcado por afinidad; y

(ii) medición de la unión del anticuerpo diseñado con cisteína y marcado por afinidad a un medio de captura.

Diseño con cisteína de las variantes de 10F4 IgG anti-CD22

La cisteína se introdujo en la posición 118 de la cadena pesada (numeración EU) (equivalente a la posición 121 de la cadena pesada, numeración secuencial) a los anticuerpos anti-CD22 monoclonales parentales quiméricos de longitud completa por medio de los métodos de diseño con cisteína que se describen en la presente memoria descriptiva.

El anticuerpo parental "std anti-CD22 Hu 10F4v3 Fc" (secuencia de cadena pesada: identificador de secuencia n.°: 88, secuencia de cadena ligera: identificador de secuencia n.°: 87, Figura 5B) se diseñó con cisteína para proporcionar "A118C tio hu anti-CD22 10F4v3" (secuencia de cadena pesada: identificador de secuencia n.°: 92, secuencia de cadena ligera: identificador de secuencia n.°: 91, Figura 17), "S400C tio hu anti-CD22 10F4v3" (secuencia de cadena pesada: identificador de secuencia n.°: 93, secuencia de cadena ligera: identificador de secuencia n.°: 91, Figura 17), o "V205C tio anti-CD22 10F4v3" (secuencia de cadena pesada: identificador de secuencia n.°: 88, secuencia de cadena ligera: identificador de secuencia n.°: 91, Figuras 5B y 17).

Estos anticuerpos monoclonales diseñados con cisteína se expresaron en células de ovarios de hámster chino (CHO) por medio de la fermentación transitoria en medios que contienen 1 mM de cisteína.

Anticuerpos anti-CD22 diseñados con cisteína y marcados

Los anticuerpos anti-CD22 diseñados con cisteína pueden ubicarse específicamente o acoplarse eficazmente con un reactivo del tiol. El reactivo del tiol puede ser un reactivo enlazador multifunctional, un reactivo de captura, es decir con afinidad, marcado (por ejemplo, un reactivo enlazador de biotina), una marca de detección (por ejemplo, un reactivo fluoróforo), un reactivo de inmovilización de la fase sólida (por ejemplo, SEPHAROSE™, polistiereno o vidrio) o un enlazador a fármaco intermedio. Un ejemplo de reactivo del tiol es la N-etilmaleimida (NEM). En una forma de realización ejemplar, la reacción de un ThioFab con un reactivo enlazador de biotina proporciona un ThioFab biotinilado por medio del cual se puede detectar y evaluar la presencia y la reactividad del residuo diseñado con cisteína. La reacción de un ThioFab con un reactivo enlazador multifuncional proporciona un ThioFab un enlazador funcionalizado que puede reaccionar aún más con un reactivo de fracciones de fármacos u otra etiqueta. La reacción de un ThioFab con un enlazador a fármaco intermedio genera un conjugado de ThioFab con fármacos.

Los métodos ejemplares descritos en la presente memoria descriptiva pueden aplicarse en general a la identificación y producción de anticuerpos, y más generalmente, a otras proteínas mediante la aplicación de las fases de diseño y detección descritas en la presente memoria descriptiva.

Dicho método puede aplicarse a la conjugación de otros reactivos del tiol en la que el grupo reactivo es, por ejemplo, una maleimida, 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). El reactivo del tiol puede ser una fracción de fármaco, un fluoróforo tal como un tinte fluorescente como la fluoresceína o rodamina, un agente quelante de metales para la radioterapia o el 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 isómeros del polietilenglicol, un péptido que se une a un tercer componente u otro carbohidrato o agente lipofílico.

Utilización de anticuerpos anti-CD22 diseñados con cisteína

Los anticuerpos anti-CD22 diseñado con cisteína y sus conjugados pueden utilizarse como agentes de diagnóstico o terapéuticos. La presente invención proporciona además métodos para prevenir, controlar, tratar o mejorar uno o más síntomas asociados a un trastorno relacionado con las células B. Particularmente, la presente invención proporciona métodos para prevenir, controlar, tratar o mejorar uno o más síntomas asociados a un trastorno de proliferación celular, como el cáncer, por ejemplo, linfoma, linfoma no Hogkins (NHL), NHL agresivo, NHL agresivo recurrente, NHL indolente recurrente, NHL refractario, NHL indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. La presente invención además proporciona métodos para diagnosticar un trastorno relacionado con el CD22 o una predisposición a desarrolar dicho trastorno, así como también los métodos para identificar anticuerpos y fragmentos de unión a antígeno de anticuerpos que preferentemente se unen a polipéptidos CD22 asociados a las células B.

Otra forma de realización de la presente invención está destinada a la utilización de un anticuerpo anti-CD22 diseñado con cisteína para la preparación de un medicamento útil en el tratamiento de una enfermedad que responde a un trastorno asociado con las células B.

Anticuerpos conjugados con fármacos diseñados con cisteína (conjugados de anticuerpo-tio con fármacos)

Otro aspecto de la invención es un compuesto de conjugados de anticuerpos con fármacos que comprende un anticuerpo anti-CD22 diseñado con cisteína (Ab) y un grupo del fármaco auristatina (D) en la que el anticuerpo diseñado con cisteína se une por medio de uno o más aminoácidos sin cisteína y de una porción de enlazador (L) a D, el compuesto que posee Fórmula I.

$Ab-(L-D)_p$ I

donde p equivale a 1, 2, 3 ó 4 y en donde el anticuerpo diseñado con cisteína se prepara mediante un proceso que involucra la sustitución de uno o más residuos de aminoácidos de un anticuerpo anti-CD22 parental por uno o más aminoácidos sin cisteína.

La Figura 10 muestra las formas de realización de conjugados de un anticuerpo anti-CD22 diseñado con cisteína con fármacos (ADC), donde una fracción del fármaco auristatina 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).

Entre las posibles ventajas de los conjugados de anticuerpos anti-CD22 diseñados con cisterna con fármacos se pueden mencionar: mayor seguridad (mayor índice terapéutico), parámetros de farmacocinética mejorados, se conservan los enlaces disulfuros de las inter-cadenas del anticuerpo que pueden estabilizar el conjugado y conservar su conformación de unión activa, se definen los sitios de conjugación de fármacos y la preparación de conjugados de anticuerpos diseñados con cisterna con fármacos a partir de la conjugación de anticuerpos diseñados con cisteína con reactivos enlazadores de fármacos permite elaborar un producto más homogeneo.

Enlazadores

Por "enlazador", "unidad de enlace" o "enlace" 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, un enlazador se especifica como L. Un "enlazador" (L) es una fracción bifuncional o multifuncional que se puede utilizar para unir una o más fracciones de fármacos (D) y una unidad de anticuerpos (Ab) para formar un conjugado de anticuerpos con fármacos (ADC) de Fórmula I. Los conjugados de anticuerpos con fármacos (ADC) pueden prepararse convenientemente utilizando un enlazador que posee funcionalidad reactiva para unirse al fármaco y al anticuerpo. Un tiol de cisteína de un anticuerpo diseñado con cisteína (Ab) puede formar un enlace con un grupo electrofílico funcional de un reactivo enlazador, una fracción del fármaco o un enlazador a fármaco intermedio.

En un aspecto, un enlazador posee un sitio reactivo que posee un grupo electrofílico que es reactivo a una cisteína nucleofílica presente en un anticuerpo. El tiol de cisteína del anticuerpo reacciona con un grupo electrofílico en un enlazador y forma un enlace covalente a un enlazador. Los grupos electrofílicos útiles comprenden, entre otros, los grupos de maleimidas y haloacetamidas.

Los enlazadores incluyen un radical divalente, como un alquildiil, un arileno, un heteroarileno, 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.

Los anticuerpos diseñados con cisteína reaccionan con reactivos enlazadores o enlazadores a fármaco intermedios, con grupos electrofílicos funcionales como la maleimida o α-halo carbonilo, según el método de conjugación descrito en la página 766 de Klussman y col., (2004), *Bioconjugate Chemistry* 15(4):765-773 y según el protocolo del ejemplo x.

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" o "vc"), alanina-fenilalanina ("ala-phe" o "af"), 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 etilenoxi $-CH_2CH_2O$, como una o más unidades repetidoras ("EO" o "PEO"). Los componentes enlazadores adicionales son conocidos en la materia y algunos se describen en la presente memoria descriptiva.

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

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) tiene un grupo funcional que puede formar un enlace con un grupo funcional de una unidad de extensión. Los grupos funcionales útiles que pueden estar presentes en un anticuerpo, ya sea naturalmente o través de la manipulación química incluyen, entre otros, el sulfidrilo (-SH), amino, hidroxilo, carboxi, el grupo de hidróxilos anoméricos de un carbohidrato y el carboxil. En un aspecto, los grupos funcionales de los anticuerpos son los sulfidrilos o aminos. Los grupos de sulfidrilos pueden generarse mediante la reducción de un enlace disulfuro intramolecular de un anticuerpo. Alternativamente, los grupos sulfidrilos se pueden generar a partir de la reacción de un grupo amino de una fracción de lisina de un anticuerpo utilizando 2-iminotiolano (reactivo de Traut) u otro reactivo que produzca sulfidrilo. En una forma de realización, un anticuerpo (Ab) posee un grupo tiol sin cisteína 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.

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.

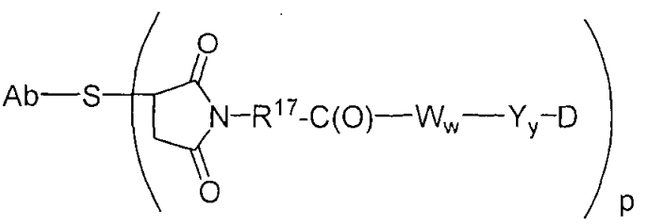
Los grupos heterociclilos incluyen un sistema de anillos en el que uno o más átomos en anillo es un heteroátomo, por ejemplo, nitrógeno, oxígeno y sulfuro. El radical heterociclo comprende de 1 a 20 átomos de carbono y de 1 a 3 heteroátomos seleccionados entre N, O, P y S. Un heterociclo puede ser un 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.

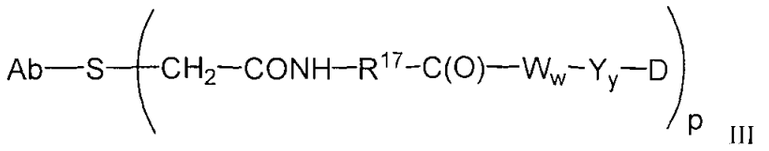
Ejemplos de heterociclos son piridil, dihidroipiridil, tetrahidropiridil (piperidil), tiazolil, tetrahidrotiofeno, tetrahidrotiofeno de azufre oxidado, pirimidinil, furanil, tienil, pirrolil, pirazolil, imidazolil, tetrazolil, benzofuranil, tianaftalenil, indolil, indolenil, quinolinil, isoquinolinil, 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.

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 monocíclicos 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.

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.

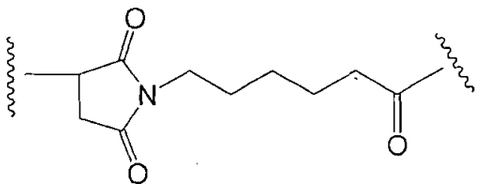


II



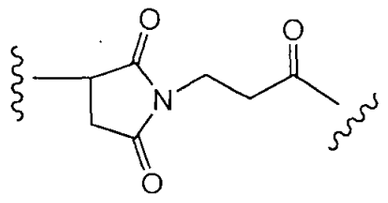
III

Una unidad de extensión ilustrativa de Fórmula II deriva de maleimida-caproila (MC) donde R^{17} es $-(CH_2)_5-$:



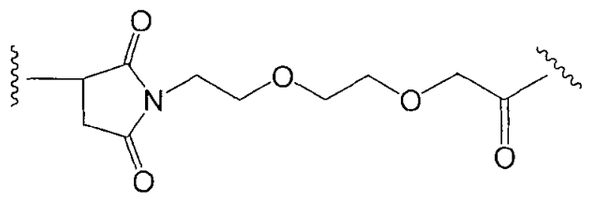
MC

Una unidad de extensión ilustrativa de Fórmula II y deriva de maleimida-propanoilo (MP) donde R^{17} es $-(CH_2)_2-$:

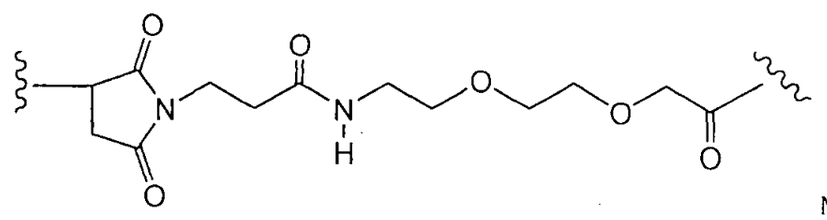


MP

Otra unidad de extensión ilustrativa de Fórmula II en la que R^{17} es $-(CH_2CH_2O)_r-CH_2-$ y r es 2:

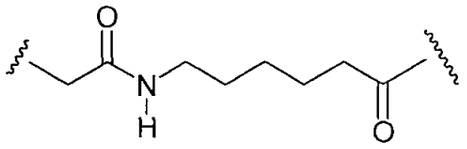


Otra unidad de extensión ilustrativa de Fórmula II en la que R^{17} es $-(CH_2)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$ donde R^b es H y cada r es 2:

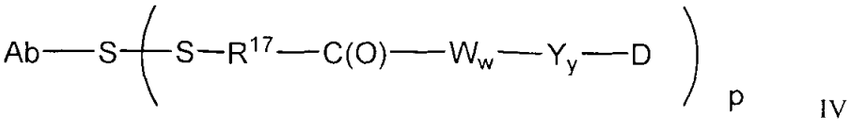


MPEG

Una unidad de extensión ilustrativa de Fórmula III en la que R^{17} es $-(CH_2)_5-$:

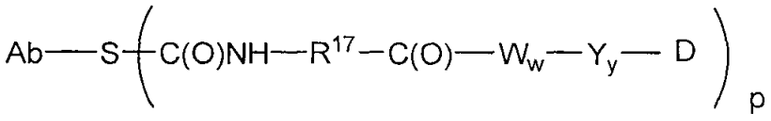


En otra forma de realización, la unidad de extensión se une al anticuerpo anti-CD22 diseñado con cisteína mediante un enlace disulfuro entre el átomo de sulfuro diseñado con cisteína del anticuerpo y un átomo de sulfuro de la unidad de extensión. Una unidad de extensión representativa de esta forma de 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.

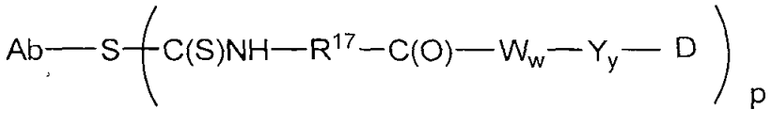


IV

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 sin cisteína 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

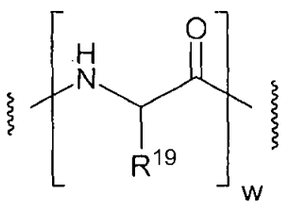
En otra forma de realización, el enlazador puede ser de tipo dendrítico para facilitar el enlace covalente de más de una fracción de fármacos a un anticuerpo mediante una fracción de enlazador multifuncional ramificado (Sun y col., (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215;

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.

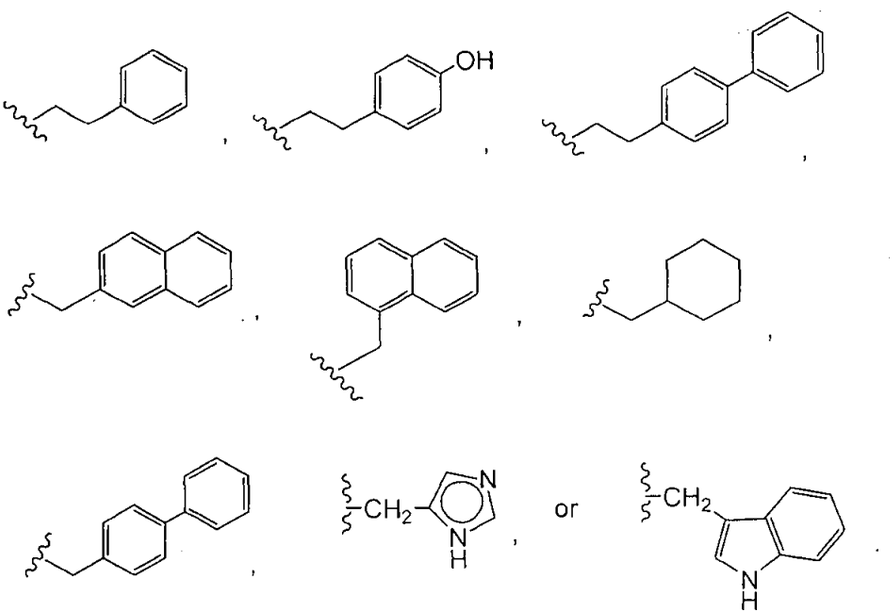
Unidad de aminoácidos

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.

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,



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.

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 (gly-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, tales como los aminoácidos menores y los análogos de aminoácidos que no se producen de forma natural, como la citrulina.

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

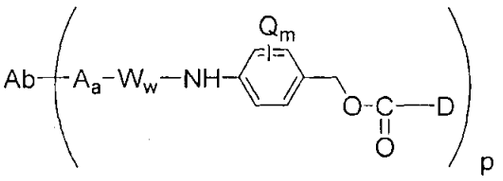
La unidad espaciadora (–Y_y–), cuando se encuentra presente (y = 1 ó 2), une una unidad de aminoácidos (–W_w–) a una fracción de fármaco (D) cuando una unidad de aminoácidos se encuentra presente (w = 1-12). Alternativamente, la unidad espaciadora une la unidad de extensión a la fracción de fármaco cuando 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.

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.

Las formas de realización ejemplares de una unidad espaciadora no autoimmoladoras son: -Gly-Gly- ; -Gly- ; -Ala-Phe- ; -Val-Cit- .

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.

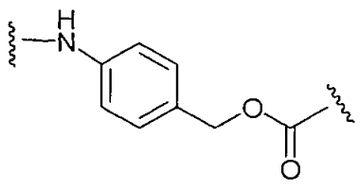
Alternativamente, un ADC que contiene una unidad espaciadora autoinmoladora puede liberar -D. En una forma de realización, -Y- es un grupo PAB unido a -W_w- a través del átomo de nitrógeno del amino del grupo PAB y está conectado directamente a -D a través de un carbonato, carbamato o grupo de éteres, donde el ADC posee la estructura ejemplar:



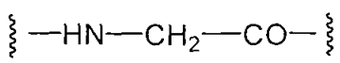
donde Q es -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -halógeno, -nitro o -ciano; m es un número entero que oscila entre 0 y 4, y p oscila entre 1 y 4.

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.

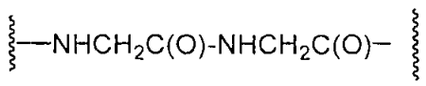
Las Fórmulas X-XII representan las unidades espaciadoras ejemplares (-Y_y-).



X



XI



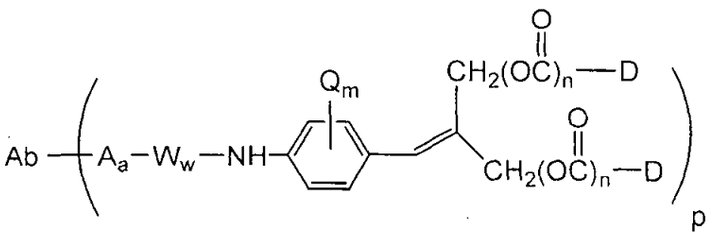
XII

Enlazadores dendríticos

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;

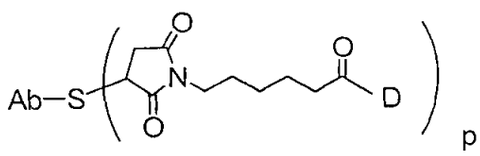
Sun y col., (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Los enlazadores dendríticos pueden incrementar la proporción molar de un fármaco a un anticuerpo, es decir, la concentración, que se relaciona con la potencia del ADC. Por lo tanto, cuando un conjugado de anticuerpos con fármacos sólo 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).

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:

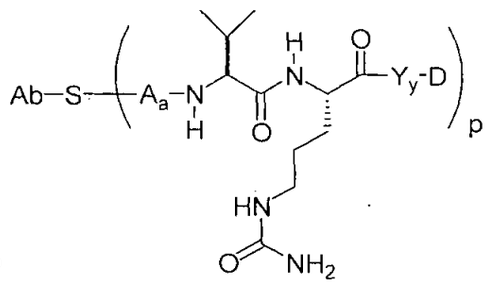


que compenda una unidad de dendrímeros de 2-(4-aminobencilideno)propano-1,3-diol (documento WO 2004/043493; de Groot y col., (2003) *Agnew. Chem. Int. Ed.* 42:4490-4494), donde Q es -C_1-C_8 alquilo, -O-(C_1-C_8 alquilo), -halógeno, -nitro o -ciano; m es un número entero que oscila entre 0 y 4; n es 0 ó 1 y p oscila entre 1 y 4.

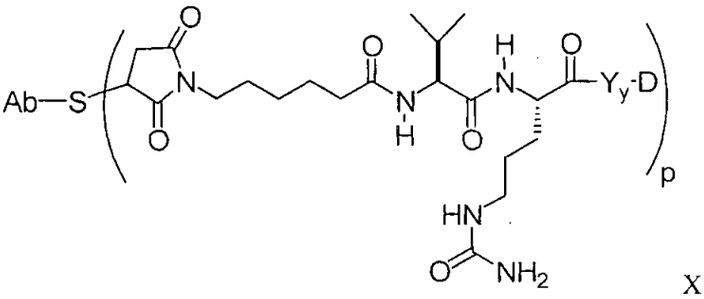
Formas de realización ejemplares de los compuestos de conjugados de anticuerpos con fármacos de Fórmula I incluyen XIIIa (MC), XIIIb (val-cit), XIIIc (MC-val-cit) y XIIId (MC-val-cit-PAB):



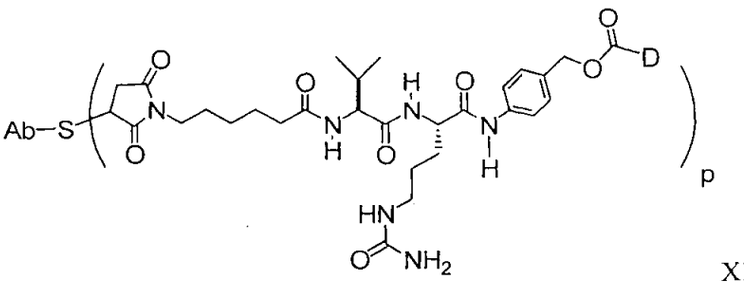
XIIIa



XIIIb

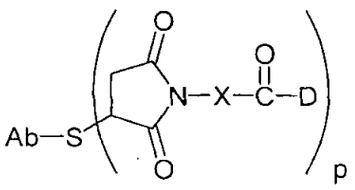


XIIIc

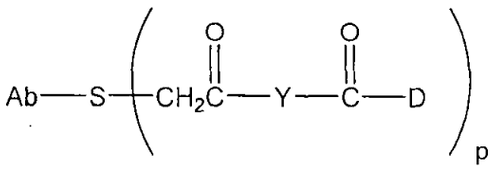


XIIId

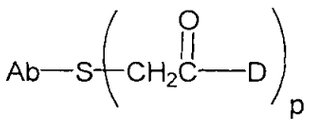
Otras formas de realización ejemplares de los compuestos de conjugados de anticuerpos con fármacos de Fórmula Ia incluyen XIV a-e:



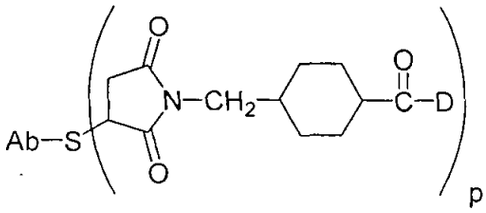
XIVa



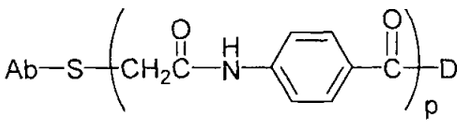
XIVb



XIVc

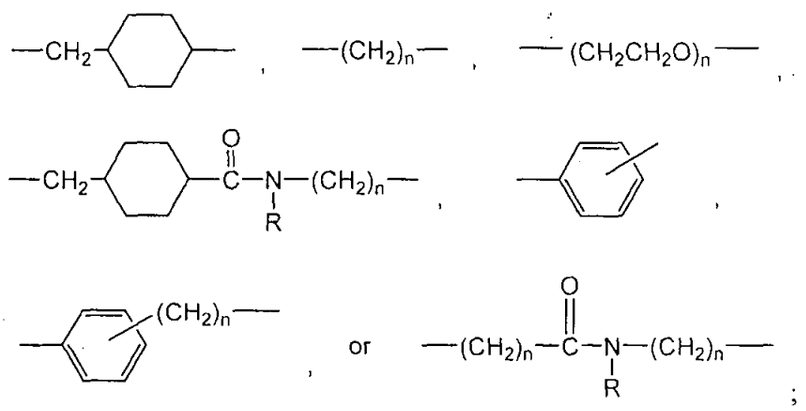


XIVd

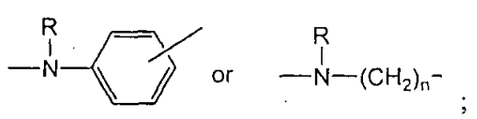


XIVe

donde X es:



Y es:



y R es independientemente H o C_1–C_6 alquilo y n oscila entre 1 y 12.

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.

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, nucleofílicos 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.

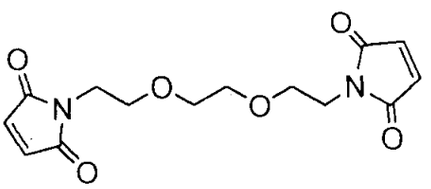
En otra forma de realización, el enlazador puede sustituirse con grupos que modularon la solubilidad o reactividad. Por ejemplo, un sustituto cargado, como el sulfonato ($-SO_3^-$) o amonio, puede incrementar la solubilidad en agua del reactivo y facilitar la reacción de unión del reactivo enlazador 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.

Reactivos enlazadores

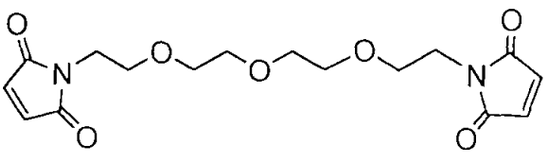
Los conjugados del anticuerpo y la auristatina se pueden realizar utilizando una variedad de reactivos enlazadores binfuncionales, como por ejemplo N-succinimidil-3-(2-piridilditio) propionato (SPDP), succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato (SMCC), iminotiolano (IT), derivados bifuncionales 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 conjugados de anticuerpos con fármacos también pueden prepararse con reactivos enlazadores: BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC y sulfo-SMPB, y SVSB (succinimidil-(4-vinilsufona)benzoato), incluyendo reactivos de bis-maleimida: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, y $BM(PEO)_4$, comercializados por Pierce Biotechnology, Inc., Departamento de Atención al cliente, P.O. Box 117, Rockford, IL. 61105 EE:UU, EE.UU 1-800-874-3723, Internacional +815-968-0747. Los reactivos de bis-maleimida permiten la unión de un grupo tiol de un anticuerpo diseñado con cisteína a una fracción de fármaco, etiqueta o enlazador intermedio que contenga tiol, de manera secuencial o simultánea. Otros grupos funcionales además de la maleimida, que son reactivos con un grupo tiol de un anticuerpo diseñado con cisteína, fracción de fármaco, etiqueta o

enlazador intermedio incluyen la iodoacetamida, bromoacetamida, vinilpiridina, disulfuro, piridil disulfuro, isocianato e isotiocianato.



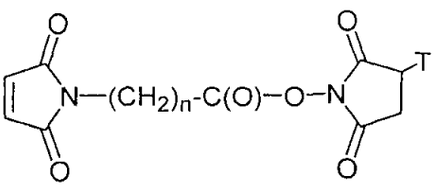
$BM(PEO)_3$



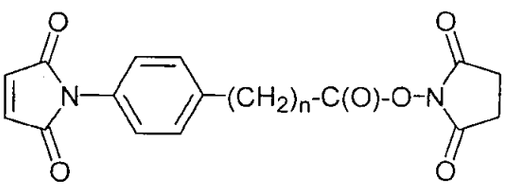
$BM(PEO)_4$

Los reactivos enlazadores útiles también se pueden obtener a través de otras fuentes comerciales, tal como Molecular Biosciences Inc. (Boulder, CO) o se pueden sintetizar según los procedimientos descritos en Toki y col., (2002) *J. Org. Chem.* 67:1866-1872; Walker, M.A. (1995) *J. Org. Chem.* 60:5352-5355; Frisch y col., (1996) *Bioconjugate Chem.* 7:180-186; la patente de EE.UU. N.º 6214345; el documento WO 02/088172; la patente de EE.UU. N.º 2003130189; la patente de EE.UU. N.º 2003096743; el documento WO 03/026577; el documento WO 03/043583 y el documento WO 04/032828.

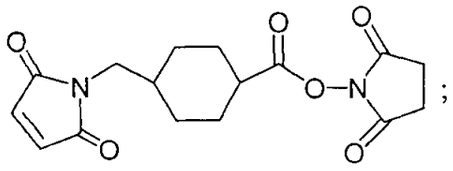
Las unidades de extensión de fórmula (IIIa) pueden introducirse en un enlazador mediante la reacción de los siguientes reactivos enlazadores con el extremo N-terminal de una unidad de aminoácidos:

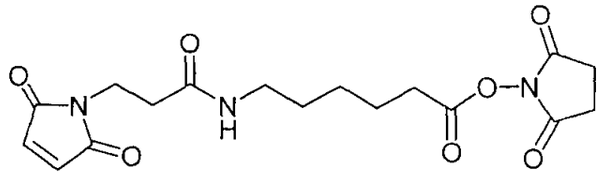


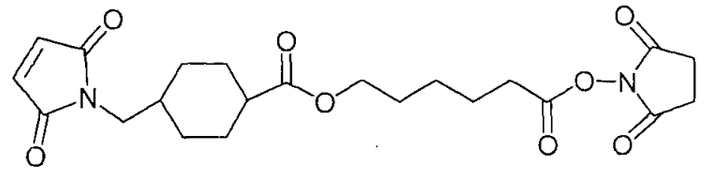
donde n es un número entero que oscila entre 1 y 10, y T es H o $-SO_3Na$;



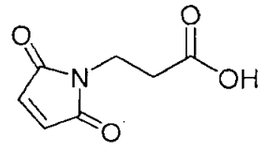
donde n es un número entero que oscila entre 0 y 3;



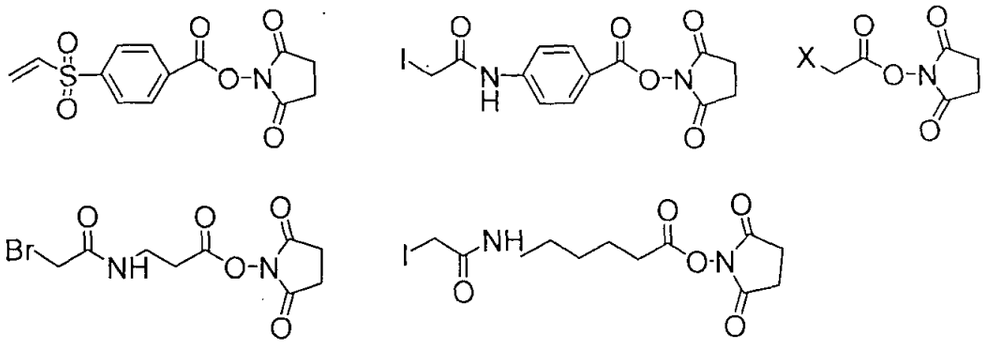




; and

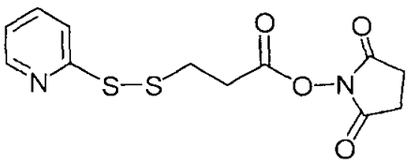


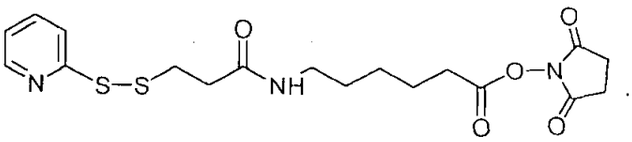
Las unidades de extensión pueden introducirse en un enlazador mediante la reacción de los siguientes reactivos bifuncionales con el extremo N-terminal de una unidad de aminoácidos.

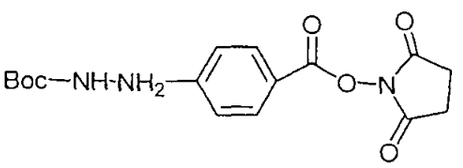


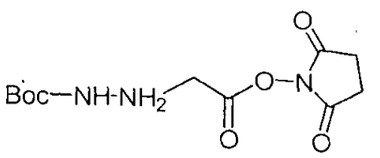
donde X es Br o I.

Las unidades de extensión de la fórmula también pueden introducirse en un enlazador mediante la reacción de los siguientes reactivos bifuncionales con el extremo N-terminal de una unidad de aminoácidos.

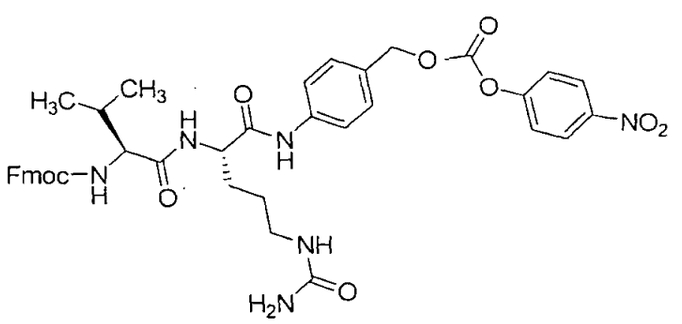




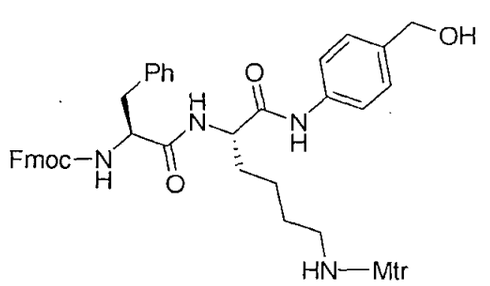




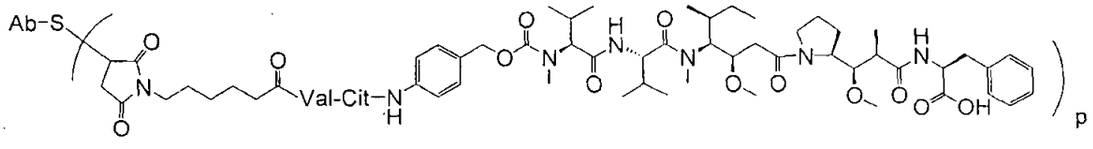
Un reactivo enlazador de dipéptidos valina-citrulina (val-cit or vc) ejemplar que posee una unidad de extensión de maleimida y un espaciador autoinmolador para-aminobencilcarbamoil (PAB) posee la siguiente estructura:



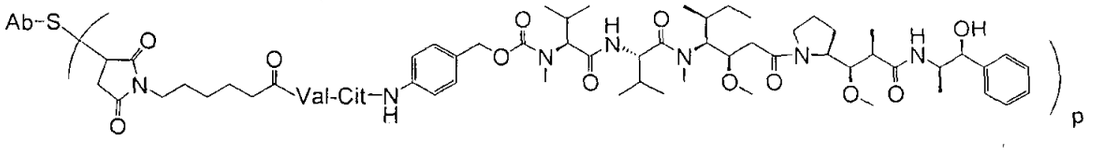
Un reactivo enlazador de dipéptidos phe-lys(Mtr, mono-4-metoxitritil) ejemplar que posee una unidad de extensión de maleimida y una unidad espaciadora autoinmoladora PAB pueden prepararse según Dubowchik y col., (1997) *Tetrahedron Letters*, 38:5257-60 y posee la siguiente estructura:



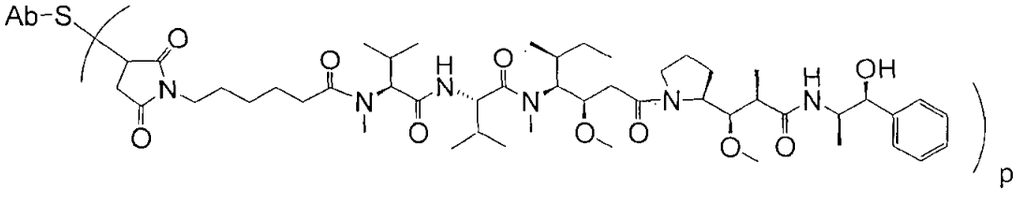
Los compuestos de conjugados de anticuerpos con fármacos ejemplares de la invención incluyen:



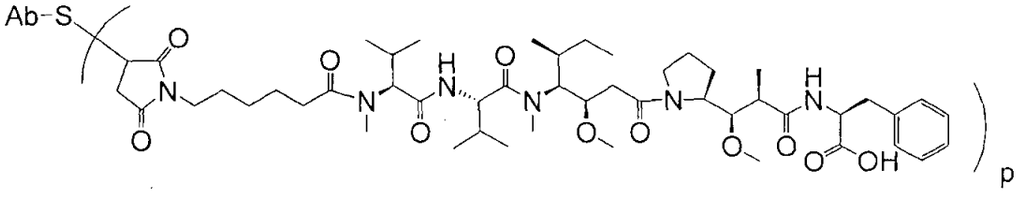
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

Donde Val es valina, Cit es citrulina, p es 1, 2, 3 ó 4 y Ab es anticuerpo anti-CD22 diseñado con cisteína.

Preparación de conjugados de anticuerpos anti-CD22 diseñados con cisteína con fármacos

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.

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.

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 anti-CD22 10F4v3 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.

La Figura 12 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 cys conjugados externamente aducidos a un cys 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.

Métodos de detección

Otra forma de realización de la presente invención se dirige a un método para determinar la presencia de un polipéptido CD22 en una muestra que se cree que contiene el polipéptido CD22. El método comprende exponer la muestra a un anticuerpo anti-CD22 diseñado con cisterna, o el conjugado de anticuerpo con fármaco del mismo, que se une al polipéptido CD22, y determinar si el anticuerpo anti-CD22 diseñado con cisterna, o el conjugado de anticuerpo con fármacos del mismo, se une al polipéptido CD22 en la muestra, en la que la presencia de dicha unión indica la presencia del polipéptido CD22 en la

muestra. Opcionalmente, la muestra puede contener células (que pueden ser cancerosas) que se sospeche que puedan expresar el polipéptido CD22. El anticuerpo anti-CD22 diseñado con cisterna, o el conjugado de anticuerpo con fármaco del mismo, que se utiliza en el método pueden opcionalmente marcarse de forma detectable, unirse a un soporte sólido, etc.

Otra forma de realización de la presente invención se dirige a un método para diagnosticar la presencia de un tumor en un mamífero. Este método comprende (a) establecer un contacto entre una muestra de prueba que contenga células del tejido obtenidas de un mamífero y un anticuerpo anti-CD22 diseñado con cisteína o, el conjugado de anticuerpo con fármaco del mismo, que se une a un polipéptido CD22, y (b) detectar la formación de un complejo entre el anticuerpo anti-CD22 diseñado con cisterna, o el conjugado de anticuerpo con fármaco del mismo, y el polipéptido CD22 en la muestra de prueba, en la que la formación de un complejo indica la presencia de un tumor en el mamífero. Opcionalmente, el anticuerpo anti-CD22 diseñado con cisterna, o el conjugado de anticuerpo con fármaco del mismo, se marca de forma detectable, se une a un soporte sólido, etc. o la muestra de prueba de las células del tejido se obtiene de un individuo que se sospecha que tienen un tumor canceroso.

Metabolitos de los conjugados de anticuerpos con fármacos

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.

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 aislan 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.

Formulaciones farmacéuticas

Administración de los conjugados de anticuerpos con fármacos, incluyendo el conjugado de anticuerpo-tio con fármacos

Los conjugados de anticuerpos con fármacos (ADC), incluyendo el conjugado de anticuerpo tio con fármacos (TDC), 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.

Para el tratamiento de estos cánceres, 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 aproximadamente 200 $\mu g/m^2$. 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.

La invención también proporciona un método para aliviar una enfermedad autoinmune, que comprende la administración al paciente con la enfermedad autoinmune de una cantidad terapéuticamente eficaz de conjugado de anticuerpo 10F4 humanizado con fármacos de cualquiera de las formas de realización precedentes. En las formas de realización preferentes, el anticuerpo se administra por vía intravenosa o subcutánea. El conjugado de anticuerpo con fármacos se administra por vía intravenosa con una dosis que varía entre aproximadamente 1 $\mu g/m^2$ y aproximadamente 100 mg/ m^2 por dosis, y en una forma de realización específica, la dosis es de entre 1 $\mu g/m^2$ y aproximadamente 500 $\mu g/m^2$. 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 el alivio de los síntomas de la enfermedad autoinmune que se trata. La administración puede continuar después de lograr el alivio de los síntomas, siempre que dicho alivio se mantenga debido a la administración continua.

La invención también proporciona un método de tratamiento de un trastorno de las células B que comprende la administración de una cantidad terapéuticamente eficaz de un anticuerpo humanizado 10F4 de cualquiera de las formas de realización precedente a un paciente que padece un trastorno de células B, tal como un trastorno proliferativo de células B (incluyendo, entre otros, el linfoma y la leucemia) o una enfermedad autoinmune, 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.

En un aspecto, la invención además proporciona formulaciones farmacéuticas que comprenden al menos un anticuerpo anti-CD22 de la invención, al menos un inmunoconjugado de éste o al menos un conjugado de anticuerpo anti-CD22 con fármacos de la invención, o todos los anteriores. En algunas formas de realización, una formulación farmacéutica comprende 1) un anticuerpo anti-CD22, un conjugado de anticuerpo anti-CD22 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-CD22 o un inmunoconjugado de éste, o ambos, y opcionalmente 2) al menos un agente terapéutico adicional.

Las formulaciones farmacéuticas que comprenden un anticuerpo, inmunoconjugado o un conjugado de anticuerpo anti-CD22 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 hidroílicos 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.

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).

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)), polilácticos (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

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.

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-CD22 o el inmunoconjugado de la invención en pacientes con trastornos proliferativos de células B, incluyendo, entre otros, el linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. 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.

Por lo general, la enfermedad o el trastorno que debe tratarse es una enfermedad hiperproliferativa, como por ejemplo, un trastorno proliferativo o un cáncer de células B, o ambos. En la presente memoria descriptiva, los ejemplos de cáncer que se tratarán incluyen, entre otros, trastornos proliferativos de células B que se seleccionan a partir del linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

El cáncer puede comprender células que expresan CD22 para que los ADC de la presente invención sean capaces de unirse a las células cancerosas. Existen diversos ensayos diagnósticos/prognósticos para determinar la expresión de CD22 en el cáncer. En una forma de realización, la sobreexpresión de CD22 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 CD22 con respecto al grado de tinción y la proporción de las células tumorales examinadas.

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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.

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-ErbB2. Otras pautas posológicas pueden ser útiles. El progreso de este tratamiento se controla fácilmente mediante técnicas y ensayos convencionales.

Terapia combinada

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 segundo componente con propiedades antineoplásicas. El segundo compuesto 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.

El segundo compuesto 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.

En un aspecto, el primer compuesto es un ADC anti-CD22 de la invención y el segundo compuesto es un anticuerpo anti-CD20 (ya sea un anticuerpo desnudo o un ADC). En una forma de realización del

segundo compuesto es el anticuerpo anti-CD20 rituximab (Rituxan®) o 2H7 (Genentech, Inc., South San Francisco, CA). Otros anticuerpos útiles para inmunoterapia combinada con los ADC anti-CD22 de la invención incluyen, entre otros, los anti-VEGF (por ejemplo, Avastin®).

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-CD20 (por ejemplo, Rituxan®) 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.

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.

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.

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

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.

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-CD22 e inmunoconjugados

Métodos diagnósticos y métodos de detección

En un aspecto, los anticuerpos anti-CD22 y los inmunoconjugados de la invención son útiles para detectar la presencia de CD22 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 CD22 a niveles más altos que en otros tejidos, por ejemplo, células B o tejidos asociados a las células B, o ambos.

En un aspecto, la invención proporciona un método de detección de la presencia de CD22 en una muestra biológica. En ciertas formas de realización, el método comprende establecer un contacto de la muestra biológica con un anticuerpo anti-CD22 en condiciones que faciliten la unión del anticuerpo anti-CD22 a CD22 y detectar si se ha formado un complejo entre el anticuerpo anti-CD22 y CD22.

En un aspecto, la invención proporciona un método para diagnosticar un trastorno asociado a un aumento de la expresión de CD22. En ciertas formas de realización, el método supone poner en contacto una célula de prueba con un anticuerpo anti-CD22; determinar el nivel de expresión (bien cuantitativo o cualitativo) de CD22 por la célula de prueba mediante la detección de la unión del anticuerpo anti-CD22 a CD22; y comparar el nivel de expresión de CD22 por la célula de prueba con el nivel de expresión de CD22 por una célula de control (por ejemplo, una célula normal del mismo origen tisular que la célula de prueba o una célula que exprese CD22 a niveles similares a dicha célula normal), donde un nivel más alto de expresión de CD22 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 CD22. 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 CD22. En ciertas formas de realización, el trastorno es un trastorno de proliferación celular, como un cáncer o un tumor.

Los trastornos proliferativos celulares ejemplares que pueden diagnosticarse mediante un anticuerpo de la invención incluyen un trastorno de células B o un trastorno proliferativo de células B, o ambos, incluyendo, entre otros, linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

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-CD22 a CD22 expresado en la superficie de una célula o en una preparación de membrana obtenida de una célula que exprese CD22 en su superficie. En ciertas formas de realización, el método comprende establecer un contacto de una célula con un anticuerpo anti-CD22 en condiciones que faciliten la unión del anticuerpo anti-CD22 a CD22 y detectar si se ha formado un complejo entre el anticuerpo anti-CD22 y CD22 en la superficie celular. Un ensayo ejemplar para detectar la unión de un anticuerpo anti-CD22 a CD22 que exprese CD22 en la superficie de una célula es un ensayo "FACS".

Se pueden utilizar otros métodos para detectar la unión de anticuerpos anti-CD22 a CD22. 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).

En ciertas formas de realización, los anticuerpos anti-CD22 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.º 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.

En ciertas formas de realización, los anticuerpos anti-CD22 están inmovilizados en una matriz insoluble. La inmovilización supone separar el anticuerpo anti-CD22 de cualquier CD22 que permanezca libre en solución. Tradicionalmente, esto se consigue bien insolubilizando el anticuerpo anti-CD22 antes del procedimiento de ensayo, o bien mediante adsorción en una superficie o matriz insoluble al agua (Bennich y col., patente de EE.UU. N°. 3.720.760), o bien mediante acoplamiento covalente (por ejemplo, usando reticulación con glutaraldehído), o bien mediante insolubilización del anticuerpo anti-CD22

después de la formación de un complejo entre el anticuerpo anti-CD22 y CD22, por ejemplo, mediante inmunoprecipitación.

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-CD22 o en adición a él.

Métodos terapéuticos

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-CD22 o un inmunoconjugado del mismo en condiciones que faciliten la unión del inmunoconjugado a CD22.. "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 B. En ciertas formas de realización, la célula es un xenotrasplante, por ejemplo, como se ejemplifica en la presente memoria descriptiva.

En un aspecto, un anticuerpo o inmunoconjugado de la invención se usa para tratar o prevenir un trastorno proliferativo de células B. En ciertas formas de realización, el trastorno de proliferación celular se asocia a un aumento de la expresión o actividad, o ambas, de CD22. Por ejemplo, en ciertas formas de realización, el trastorno proliferativo de células B se asocia a un aumento de la expresión de CD22 en la superficie de una célula B. En ciertas formas de realización, el trastorno proliferativo de células B es un tumor o cáncer. Los ejemplos de trastornos proliferativos de células B que se tratan mediante anticuerpos o inmunoconjugados de la invención incluyen, entre otros, linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

En un aspecto, la invención proporciona métodos para el tratamiento de un trastorno proliferativo de células B que comprende la administración a un sujeto de una cantidad eficaz de un anticuerpo anti-CD22 o un inmunoconjugado de éste. En ciertas formas de realización, un método para el tratamiento de un trastorno proliferativo de células B supone la administración a un sujeto de una cantidad eficaz de una formulación farmacéutica que comprenda un anticuerpo anti-CD22 o inmunoconjugado anti-CD22 y, opcionalmente, al menos un agente terapéutico adicional, como los que se proporcionan más adelante. En

ciertas formas de realización, un método para el tratamiento de un trastorno de proliferación celular supone la administración a un sujeto de una cantidad eficaz de una formulación farmacéutica que comprenda 1) un inmunoconjugado que contenga un anticuerpo anti-CD22 y un agente citotóxico, y, opcionalmente, 2) al menos un agente terapéutico adicional, como los que se proporcionan más adelante.

En un aspecto, al menos algunos de los anticuerpos o inmunoconjugados de la invención se pueden unir a CD22 de especies distintas a la humana. Por consiguiente, los anticuerpos o inmunoconjugados de la invención pueden usarse para unirse a CD22, por ejemplo, en un cultivo celular que contenga CD22, en humanos o en otros mamíferos que tengan un CD22 con el que un anticuerpo o inmunoconjugado de la invención reaccione (por ejemplo, chimpancé, babuino, tití, macaco de Java y macaco de la India, cerdo o ratón). En una forma de realización, un anticuerpo anti-CD22 o inmunoconjugado puede utilizarse para que CD22 actúe sobre las células B al poner en contacto un anticuerpo o inmunoconjugado con CD22 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, el CD22 es CD22 humano.

En una forma de realización, un anticuerpo anti-CD22 o un inmunoconjugado se puede utilizar en un método para unir CD22 en un sujeto que padezca un trastorno asociado a un aumento de la expresión y/o actividad de CD22 y dicho método comprende la administración al sujeto del anticuerpo o inmunoconjugado de forma que, en el sujeto, se una el CD22. En una forma de realización, el anticuerpo o inmunoconjugado unido se internaliza en las células B que expresan CD22. En una forma de realización, CD22 es CD22 humano y el sujeto es un humano. Alternativamente, el sujeto puede ser un mamífero que exprese CD22 al que se une un anticuerpo anti-CD22. Además, el sujeto puede ser un mamífero al que se le ha introducido CD22 (por ejemplo, mediante la administración de CD22 o mediante la expresión de un trasgén que codifique CD22).

Un anticuerpo anti-CD22 o un inmunoconjugado puede administrarse a un humano con fines terapéuticos. Además, un anticuerpo anti-CD22 o un inmunoconjugado puede administrarse a mamíferos no humanos que expresan CD22 con el que el anticuerpo reaccione (por ejemplo, primate, 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).

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-CD20 (por ejemplo, Rituxan®) o anti-VEGF (por ejemplo, Avastin®), donde la terapia combinada es útil en el tratamiento de cánceres o trastornos de células B, o ambos, como por ejemplo, trastornos proliferativos de células B, incluyendo, linfoma, linfoma no Hogkins (NHL), NHL agresivo, NHL agresivo recurrente, NHL indolente recurrente, NHL refractario, NHL indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

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.

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.

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.

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

Los anticuerpos anti-CD22 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

En un aspecto, se proporcionan ensayos para la identificación de anticuerpos anti-CD22 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*.

En ciertas formas de realización, se comprueba la capacidad de un anticuerpo anti-CD22 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).

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.

En un aspecto, se comprueba la capacidad de un anticuerpo anti-CD22 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 3×10^6 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.

En un aspecto, se comprueba la capacidad de un anticuerpo anti-CD22 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).

Las células para uso en cualquiera de los ensayos *in vitro* anteriores incluyen células o líneas celulares que expresan naturalmente CD22 o que se han diseñado para expresar CD22. Estas células incluyen células tumorales que sobreexpresan CD22 con respecto a células normales del mismo origen

tisular. Estas células también incluyen líneas celulares (incluidas líneas celulares tumorales) que expresan CD22 y líneas celulares que normalmente no expresan CD22 pero han sido transfectadas con ácido nucleico que codifica CD22.

En un aspecto, se comprueba la capacidad de un anticuerpo anti-CD22 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-CD22 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. Estas células que son útiles para preparar modelos xenotransplantados incluyen leucemia humana y líneas celulares de linfomas, que incluyen, entre otras, las células BJAB-luc (una línea celular de linfoma de Burkitt EBV-negativa transfectada con el gen indicador de luciferasa), células Ramos (ATCC, Manassas, VA, CRL-1923), células Raji (ATCC, Manassas, VA, CCL-86), células SuDHL-4 (DSMZ, Braunschweig, Germany, AAC 495), células DoHH2 (véase Kluin-Neilemans, H.C. y col., *Leukemia* 5:221-224 (1991), y Kluin-Neilemans, H.C. y col., *Leukemia* 8:1385-1391 (1994)), células Granta-519 (véase Jadayel, D.M. y col., *Leukemia* 11(1):64-72 (1997)). 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

En un aspecto, se prueba la actividad de unión de un anticuerpo anti-CD22 al antígeno. Por ejemplo, en ciertas formas de realización, se comprueba la capacidad de un anticuerpo anti-CD22 para unirse a CD22 expresado en la superficie de una célula. Para realizar esta prueba puede utilizarse un ensayo FACS.

En un aspecto, los ensayos de competición pueden utilizarse para identificar un anticuerpo monoclonal que compite con un anticuerpo murino 10F4.4.1, un anticuerpo humanizado 10F4v1, un anticuerpo humanizado 10F4v3 o un anticuerpo murino 5E8.1.8, o todos los anteriores, para que se unan a CD22. En ciertas formas de realización, este anticuerpo competitivo se une al mismo epitopo (por ejemplo, un epitopo lineal o conformacional) que se une mediante un anticuerpo murino 10F4.4.1, un anticuerpo humanizado 10F4v1, un anticuerpo humanizado 10F4v3 o un anticuerpo murino 5E8.1.8., o

todos los anteriores. 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.

En un ensayo de competición ejemplar, un CD22 inmovilizado se incuba en una solución que comprenda un primer anticuerpo marcado que se una a CD22 (por ejemplo, un anticuerpo murino 10F4.4.1, un anticuerpo humanizado 10F4v1, un anticuerpo humanizado 10F4v3 o un anticuerpo murino 5E8.1.8.) y un segundo anticuerpo sin marcar del que se comprueba su capacidad para competir con el primero para su unión a CD22. El segundo anticuerpo puede estar presente en un sobrenadante de hibridoma. Como control, se incuba un CD22 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 CD22, se elimina el anticuerpo no unido sobrante y se mide la cantidad de marcador asociada a CD22 inmovilizado. Si la cantidad de marcador asociada a CD22 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 CD22. En ciertas formas de realización, un CD22 inmovilizado está presente en la superfice de una célula o en una preparación de membrana obtenida a partir de una célula que exprese CD22 en su superficie.

En un aspecto, los anticuerpos purificados anti-CD22 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.

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.º 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

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 del anticuerpo monoclonal murino CD22 antihumano

Se prepararon anticuerpos monoclonales murinos capaces de unir específicamente CD22 humano. Los panículos de las patas de ratones hembra BALB/c de seis semanas se inmunizaron con el dominio extracelular CD22 humano marcado con 8 his que carece de los dominios 3 y 4 (identificador de secuencia n.º: 30 (ECD) más la secuencia GRAHHHHHHHH en el extremo C-terminal) o el dominio extracelular CD22 marcado con 8 his que comprende los dominios del 1 al 7 (identificador de secuencia n.º: 28 (ECD) más la marca de secuencia His mencionada anteriormente) en el adyuvante de Ribi. Se administraron inyecciones posteriores de la misma manera a la primera y tercera semana tras las inmunizaciones iniciales. Tres días después de la inyección final, se retiraron y analizaron los ganglios linfáticos poplíteos e inguinales y se realizó una suspensión unicelular al pasar el tejido a través de una gasa de acero. Las células se fusionaron a una proporción 4:1 con mielomas de ratón tal como P3X63-Ag8.653 (ATCC CRL 1580) con alto contenido de glucosa (DMEM) con un 50% p/v de polietilenglicol 4000. A continuación, las células fusionadas se colocaron sobre una placa a una densidad de 2x105 por pocillo en placas de cultivo de tejido de 96 pocillos. Después de 24 horas, se agregó un medio HAT selectivo (hipoxantina/aminopterina/timidina, Sigma, #H0262). Quince días después de la fusión, se evaluaron los

sobrenadantes de células de crecimiento para comprobar la presencia de anticuerpos específicos para el CD22 humano mediante un ensayo de inmunoabsorción enzimática (ELISA).

Se seleccionaron anticuerpos monoclonales murinos antihumanos CD22 10F4.4.1 (mu 10F4) y 5E8.1.8 (mu 5E8) para someterlos a un estudio basado en ensayos con células y ensayos en placas que indicaron que los anticuerpos se unen específicamente al CD22 humano. Los ensayos se describen en los siguientes párrafos.

Ensayo ELISA: La detección del anticuerpo anti-CD22 mediante el ensayo ELISA se realizó de la siguiente manera; todas las incubaciones se realizan a temperatura ambiente. Las placas de prueba (pocillos Nunc) se recubrieron durante 2 horas con CD22 purificado en 50 mM de tampón de carbonato de sodio, pH 9,6, después se bloquearon con 0,5% de albúmina de suero bobino en solución salina fosfatada (PBS) durante 30 minutos, y se lavaron cuatro veces con PBS con un 0,05% de Tween 20 (PBST). Se agregaron los sobrenadantes de anticuerpos, se incubaron durante dos horas con agitación y después se lavaron cuatro veces con PBST. Las placas se desarrollaron al agregar 100 µl por pocillo de una solución que contiene 10 mg de o-fenilendiamina dihidrocloruro (Sigma, #P8287) y 10 ml de una solución al 30% de peróxido de hidrógeno en 25 ml de tampón de citrato de fosfato, pH 5,0, y se incuban durante 15 minutos. La reacción se detiene al agregar 100 µl por pocillo de ácido sulfúrico de 2,5 M. Los datos se obtienen mediante la lectura de las placas en un lector de placas ELISA automatizado a una absorbancia de 490 nm.

Ejemplo 2: Ensayo FACS para analizar los anticuerpos monoclonales CD22 antihumanos (MAbs).

Las células CHO que expresan CD22 humano en la superficie se incubaron con un sobrenadante del hibridoma anti-CD22 en 100 µl de tampón FACS (0,1% de BSA, 10 mM de azida sódica en PBS, pH 7,4) durante 30 minutos a 4 °C y se lavaron una vez con el tampón FACS. La cantidad de unión a anti-CD22 se determinó mediante la incubación de una alícuota de la mezcla de anticuerpos y células con un anti-IgG de ratón en cabra o conejo conjugado con FITC policlonal (Accurate Chem. Co., Westbury, NY) (para anticuerpos de prueba murinos) o IgG antihumano de cabra o conejo (para anticuerpos humanizados) durante 30 minutos a 4 °C y se lavó tres veces con el tampón FACS.

Ejemplo 3: Preparación de anticuerpos anti-CD22 humanizados

Se generaron anticuerpos 10F4 humanizados en donde los residuos de aminoácidos de la región hipervariable (HVR) (llamadas indistintamente regiones determinantes de la complementariedad o CRD) se modificaron a través de mutagénesis dirigida al sitio (Kunkel y col., *Methods Enzymol.* (1987), 154:367-382) para obtener dos variantes, 10F4v1 humanizado y 10F4v2 humanizado (también llamados en la

presente memoria descriptiva "10F4v1," "hu10F4v1," "10F4v2" o "hu10F4v2," respectivamente). Una tercera versión, 10F4v3 humanizado ("10F4v3" o "hu10Fv3"), que se utiliza en algunos estudios descritos en la presente memoria descriptiva, tiene las mismas secuencias de aminoácidos de la cadena ligera y pesada para la proteína madura que hu10F4v2, pero comprende una secuencia de señal diferente en el vector utilizado para la expresión de la proteína.

La humanización del anticuerpo murino 10F4 se realizó tal como se detalla en la presente memoria descriptiva. En resumen, las regiones hipervariables de las cadenas ligera y pesada del 10F4 murino se clonaron en secuencias de estructuras de consenso modificadas para generar las secuencias de aminoácidos de regiones variables de la cadena ligera y pesada, tal como se muestran en la Figura 2A y 2B. Las secuencias de estructuras de la cadena ligera y pesada alternativas que pueden utilizarse como secuencias de estructuras de anticuerpos de la invención se muestran en las Figuras 3 y 4.

Para la humanización del anticuerpo 10F4 se utilizó un vector (pV0350-2B) fagémido que representa un Fab-g3 monovalente con dos estructuras de lectura abierta bajo el control del promotor *pho*A, particularmente como se describe en Lee y col., *J. Mol. Biol.* 340:1073-93 (2004). La primera estructura de lectura abierta comprende la secuencia de señal termoestable STII *E. coli* para la secreción de proteínas fusionada con los dominios VL y CH1 de la secuencia de la cadena ligera del aceptor. La segunda estructura de lectura comprende la secuencia de señal STII fusionada con los dominios VH y CH1 de la secuencia de la cadena pesada del aceptor seguido de una proteína P3 de revestimiento menor truncada.

Los dominios VH y VL del 10F4 murino (identificador de secuencia n.°: 89 y 90, respectivamente) se alinearon con el dominio de la VH de consenso del subgrupo III humano (huIII) (identificador de secuencia n.°: 24) y el dominio kappa1 de consenso humano (huK1) (identificador de secuencia n.°: 25), respectivamente. Las secuencias de aminoácidos de las regiones hipervariables (HVR, denominadas indistintamente en la presente memoria descriptiva regiones de determinación de complementariedad (CDR)) del MAb 10F4 murino CD22 antihumano se insertaron en las secuencias de estructuras de consenso tal como se indica a continuación. Las HVR de la cadena ligera (HVR-L1 (posiciones 24-34 de la numeración de Kabat), HVR-L2 (posiciones 50-56 de la numeración de Kabat) y HVR-L3 (posiciones 89-97 de la numeración de Kabat)) del anticuerpo murino 10F4 se diseñaron en una secuencia de estructuras de consenso del anticuerpo kappa I humano (huKI) para producir la cadena ligera humanizada 10F4v1 (identificador de secuencia n.°: 2B). Las HVR de la cadena pesada (HVR-H1 (posiciones 26-35 de la numeración de Kabat), HVR-H2 (posiciones 49-65 de la numeración de Kabat) y HVR-H3 (posiciones 95-102 de la numeración de Kabat)) del anticuerpo murino 10F4 se diseñaron en un dominio de VH de

consenso del subgrupo III humano (humIII) modificado que difiere de la secuencia humIII en tres posiciones: R71A, N73T y L78A se utilizaron (véase Carter y col., *Proc. Natl. Acad. Sci. USA* 89:4285 (1992)) para producir la región variable de la cadena pesada 10F4v1 humanizada (identificador de secuencia n.º: 16, Figura 2A). La ingeniería genética de las HVR en las estructuras del aceptor se realizó mediante mutagénesis según el método de Kunkel utilizando un oligonucleótido individual para cada región hipervariable. La secuencia de cada clon se determinó mediante técnicas estándares de secuenciación de ADN. Las regiones hipervariables y estructurales que se muestran en las Figuras 2A y 2B se enumeran según la numeración de Kabat (Kabat y col., (1991), mencionado anteriormente). Las cadenas ligera y pesada se secuenciaron y las secuencias de aminoácidos de las regiones variables (incluyendo las HVR y las regiones estructurales (FR)) de huKI, humIII, murino 10F4, 10F4v1 humanizado y 10F4v2 humanizado se muestran en Figuras 2A y 2B El anticuerpo humanizado 10F4v3 tiene una secuencia de aminoácidos idéntica al 10F4v2.

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.

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.

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)

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.

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.

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.

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).

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.

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 huésped 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.

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 huésped apropiada.

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.

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.

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'.

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.

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.

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.

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.

Ejemplo 4: Determinación de la secuencia de región variable

Las secuencias de aminoácidos y ácido nucleico de los anticuerpos monoclonales murinos 10F4 humanizados se determinó mediante procedimientos estándares. Se extrajo el ARN total de las células de hibridoma que producen los anticuerpos monoclonales 10F4.4.1 de ratón CD22 antihumano, mediante el Mini Kit RNeasy® (Qiagen, Alemania). El dominio variable ligero (VL) y el dominio variable pesado (VH) se amplificaron usando RT-PCR con los cebadores degenerados. Los cebadores anversos eran específicos para la secuencia de aminoácidos N-terminal de las regiones VL y VH del anticuerpo. 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 conserva en gran medida entre las especies. Las VL y VH amplificadas se clonaron en un vector de expresión celular de mamífero pRK (Shields y col., *J. Biol. Chem.* 276:659-04 (2000)). La secuencia de polinucleótidos en las inserciones se determinó mediante los métodos de secuenciación habituales. Las secuencias de

aminoácidos del 10F4 murino quimérico y el 10F4v1 humanizado, y las regiones variables de la cadena ligera y pesada del 10F4v2 humanizado se muestran en las Figuras 2A y 2B.

El 10F4v1 humanizado se modificó aún más en la posición 28 del HVR-L1 (N28) (identificador de secuencia n.°: 9) (véase Figura 2B). El residuo de asparragina en esa posición se reemplazó por un residuo de valina (N28V) para generar un HVR-L1 (identificador de secuencia n.°: 10) para las variantes hu10F4v2 y hu10F4v3, que muestran una afinidad de unión mejorada. Dichas variantes comprenden las mismas secuencias de dominio constante y variable del anticuerpo maduro y difieren sólo en una secuencia de señal que no se encuentra en el anticuerpo maduro de la invención.

Las modificaciones adicionales de la secuencia de aminoácidos se generaron en uno o ambos aminoácidos Asn28 (N28) o Asn30 (N30) de la región hipervariable HVR-L1 (véase Figura 2B) de hu10F4v1. Dado que N28 y N30 son posibles sitios de deaminación, se evaluaron los cambios de los aminoácidos en estos sitios. Por ejemplo, la asparragina en la posición 28 (N28) se reemplazó alternativamente con A, Q, S, D, V o I, y la asparragina en la posición 30 (N30) se reemplazó alternativamente con A o Q. Los cambios de la secuencia de aminoácidos en el dominio HVR-L1 según la invención se proporcionan en la Tabla 2, junto con sus afinidades de unión tal como se evaluaron mediante análisis de competición en un ensayo ELISA de fagos (IC 50) utilizando procedimientos estándares.

Tabla 2

Variantes de sustitución del anticuerpo hu10F4v1

Cambios de aminoácidos en HVR-L1 Figura 2B	Identificador de secuencia de HVR-L1 n.°:	Afinidad de unión (nM)
Sin cambios (N28, N30)	9	8
N28A, N30	19	8
N28Q, N30	20	7.3
N28S, N30	21	12
N28D, N30	22	12
N28V, N30	10	7.3
N28I, N30	23	9.8
N28, N30A	32	7.7
N28, N30Q	33	10

Para la generación de versiones IgG1 humanas de logitud completa del anticuerpo 10F4 humanizado, las cadenas ligera y pesada se subclonan por separado en los plásmidos pRK descritos anteriormente (Gorman, C.M. y col., (1990), *DNA Protein Eng. Tech.* 2: 3). Los plásmidos apropiados de las cadenas ligera y pesada (dependiendo de los cambios de secuencias deseados) se cotransfeccionaron en líneas ceululares del riñón embrionario humano transformadas en adenovirus, conocidas como 293 (Graham, F.L. y col., (1977), *J. Gen. Virol.* 36: 59), mediante un procedimiento altamente eficaz (Graham y col., mencionado anteriormente, y Gorman, C.M., *Science* 221: 551). El medio cambia a un medio sin suero y las células se recolectaron diariamente durante un máximo de 5 días. Los anticuerpos se purificaron de los sobrenadantes agrupados utilizando la proteína A-Sefarosa CL-4B (Pharmacia). El anticuerpo eluído se cambia de tampón a PBS mediante filtración de gel G25, concentrado mediante ultrafiltración con un Centriprep-30 o Centricon-100 (Millipore) y almacenado a 4 °C. La concentración del anticuerpo se determina mediante ELISA de unión IgG total.

Los dominios constantes de la cadena pesada de IgG ejemplares se describen en la Figura 5A. Un dominio constante κ de la cadena ligera humana comprende, por ejemplo, RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHDVYACEVTHQGLSSPVTKSFNRGEC (identificador de secuencia n.°: 37). En la Figura 5B se muestra la secuencia de aminoácidos de longitud completa de h10F4v2, en la que las regiones constantes de las cadenas ligera y pesada están subrayadas. Los anticuerpos h10F4v1, v2 y v3 son isotipos IgG1.

Caracterización de anticuerpos anti-CD22

Ejemplo 5: Mapeo de epítopos:

Los epítopos de CD22 a los que se unen los anticuerpos 10F4.4.1 y 5E8.1.8 se determinaron de acuerdo con los siguientes procedimientos. Las secuencias CD22 carentes de varios de los siete dominios de tipo inmunoglobulina del isoforma CD22 principal (CD22 beta) se clonaron y transformaron en células para lograr la expresión estable. Por ejemplo, las variantes de CD22 carentes del dominio 1 (Δ1), el dominio 2 (Δ2) o los dominios 3 y 4 (Δ3, 4) se clonaron, se transformaron en células CHO y se expresaron en las células. Las células de control expresaron CD22 beta. Las eliminaciones se realizaron mediante el kit de reactivos QuikChange XL™ de Stratagene. La eliminación del dominio 1 se realizó mediante la eliminación de los aminoácidos del 22 al 138, la eliminación del dominio 2 se realizó mediante la eliminación de los aminoácidos del 139 al 242 y los dominios 3 y 4 eliminados estaban disponibles como isoforma CD22 alfa secundario (eliminación de aminoácidos del 241 al 417). Todos los números de los

aminoácidos se refieren a la numeración del precursor CD22 beta de longitud completa que describe Wilson, G.L. y col., (véase Figura 1 en Wilson, G.L y col., *J. Exp. Med.* 173:137-146 (1991)). La Figura 14 es un diagrama de los dominios que se eliminaron. La unión se determinó mediante citometría de flujo con un control de isotipo. La unión de 10F4.4.1 se detectó mediante Alexa 488 de IgG anti-ratón de cabra. La unión de 5E8.1.8 se detectó mediante IgG anti-ratón de cabra biotinilado más estreptavidina PE. Un efecto secundario de la unión de los anticuerpos murino 10F4.4.1 o murino 5E8.1.8 en la ausencia de los dominios ECD particulares indicaron que el anticuerpo se une a dichos dominios. Los anticuerpos 10F4.4.1 y 5E8.1.8 murinos mostraron las mismas características de unión en estas mismas condiciones. Ninguno se unió a CD22 que carezca del dominio 1 o dominio 2 y ambas se unieron a CD22 que comprenden los dominios 1 y 2, pero carecen de los dominios 3 y 4. Mediante este método se determinó que 10F4.4.1 y 5E8.1.8 se unen a los dominios 1 y 2 del CD22 humano dentro de la secuencia de aminoácidos del 22 al 240 de identificador de secuencia n.º: 27 (véase Wilson, G.L. y col., (1991), mencionado anteriormente).

Ejemplo 6: Caracterización de la afinidad de unión al antígeno soluble

La afinidad de unión del anticuerpo 10F4 murino y humanizado por el dominio extracelular (ECD) CD22 soluble se determinó mediante medición con resonancia de plasmón superficial utilizando un sistema BIACORE® 3000 (Biacore, Inc., Piscataway, NJ). Los biosensores de dextrano carboximetilado (CM5, Biacore Inc) se activaron con clorhidrato de *N*-etil-*N'*- (3-dimetilaminopropil)-carbodiimida (EDC) y *N*-hidroxisuccinimida (NHS), según las instrucciones del proveedor. Estos chips activados se recubrieron con un anticuerpo IgG1 anti-CD22 10F4 (murino o humanizado) mediante dilución a 5 µg/ml con 10mM de acetato sódico, pH 4,8, antes de aplicar la inyección a un caudal de 5µl/minuto para lograr aproximadamente 500 unidades de respuesta (RU) del anticuerpo acoplado. A continuación, se inyectó 1M de etanolamina para bloquear los grupos no reaccionados. Para mediciones cinéticas, se inyectó el doble de diluciones seriadas de antígeno soluble marcado con CD22-beta-ECD-His humano (de 7,8 nM a 500 nM aproximadamente) en PBS con 0,05% de Tween 20 a 25 °C a un caudal de 30 ul/min. Las tasas de asociación (k_{on}) y las tasas de disociación (k_{off}) se calcularon mediante un sencillo modelo de unión uno a uno de Langmuir (BIAevaluation Software versión 3.2). La constante de disociación de equilibrio (Kd) se calculó como el cociente k_{off}/k_{on}. El anticuerpo anti-CD22, RFB4, se utilizó como control (Chemicon International, Inc., Temecula, CA, catálogo N.º CBL147) Los resultados de este experimento se muestran en la Tabla 2.

TABLA 2

Afinidad de unión de anti-CD22 a CD22 humano soluble (Análisis BIACORE®)

Clon	$k_{on}/10^5$	$k_{off}/10^{-4}$	Kd (nM)
10F4 murino	0.19	2.8	15
10F4 quimérico	0.26	4.2	16
10F4v1 humanizado	0.18	3.5	19
10F4v2 humanizado	0.32	2.5	7.8
RFB4 de control	0.33	1.4	4.2

Ejemplo 7: Caracterización de la afinidad de unión al antígeno de superficie celular

La afinidad de unión del 10F4.4.1 murino y 10F4v1 y 10F4v2 humanizados para el CD22 humano y mono cinomolgus expresado en la superficie de las células CHO se examinó mediante un ensayo de competición. De manera resumida, las células CHO se expresan de manera estable en el CD22 humano (identificador de secuencia n.º: 27) o el CD22 de mono cinomolgus (identificador de secuencia n.º: 31) de longitud completa. El anticuerpo anti-CD22 (10F4v1 o v2 murino o humanizado) se yodó con el reactivo Iodogen® (^{125}I) para una actividad específica de aproximadamente 10 µCi/µg. Se realizó un ensayo de unión competitiva con células mediante un anticuerpo anti-CD22 diluído serialmente y sin marcar. Se permitió que los anticuerpos se unieran con las células durante 4 horas a 4 °C. La afinidad de unión, K_D, de los anticuerpos se determinó de acuerdo con el análisis Scatchard estándar que se realizó mediante un programa de ajuste de curva no lineal (véase, por ejemplo, Munson y col., *Anal Biochem*, 107: 220-239, 1980). Los resultados de este experimento se muestran en la Tabla 3.

TABLA 3

Afinidad de unión de 10F4 MAb al CD22 humano y de mono cinomolgus

Anticuerpo	CD22 humano Kd (nM)	CD22 del mono cinomolgus Kd (nM)
10F4.4.1 murino	2.4	2.3
Hu 10F4v1*	1.1, 1.7	1.4, 1.8
Hu 10F4v2	1.6	2.1

*ensayos repetidos

Los resultados indican que el 10F4 murino y humanizado se une al CD22 humano y de mono cinomolgus expresado en la superficie de las células CHO con una afinidad aproximadamente igual.

Ejemplo 8: Producción de conjugados de anticuerpos anti-CD22 con fármacos

Los ACD anti-CD22 se produjeron mediante conjugación de anticuerpos anti-CD22 RFB4, 5E8 murino, 10F4 murino, 10F4v1 humanizado, thioMAb 10F4v1 humanizado (thio-10F4v1), 10F4v2 humanizado y 10F4v3 humanizado con las siguientes fracciones de enlazadores a fármacos: spp-DM1, smcc-DM1, MC-vc-PAB-MMAE; MC-vc-PAB-MMAF; MC-MMAE y MC-MMAF, cuyas fracciones de fármacos y enlazadores se describen en la presente memoria descriptiva, así como en el documento WO 2004/010957, publicado el 5 de febrero de 2004, y en el documento WO2006/034488, publicado el 9 de septiembre de 2005 (las aplicaciones de las patentes 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. Otros enlazadores útiles para la preparación de ADC incluyen, entre otros, BMPEO, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC y sulfo-SMPB, y SVSB (succinimidil-(4-vinilsufona)benzoato), incluyendo reactivos de bis-maleimida: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$ y $BM(PEO)_4$.

Los ACD anti-CD22 también se producen mediante conjugación con residuos de lisina del anticuerpo. Las lisinas del anticuerpo se convierten en grupos sulfidrilos mediante, por ejemplo, reactivos de Traut (Pierce Chemical Co.), tal como se detalla en la presente memoria descriptiva. Los grupos sulfidrilos resultantes son reactivos con enlazadores y moléculas de enlazadores a fármacos para la preparación de los ACD. Alternativamente, los ACD se producen al reaccionar una lisina en un anticuerpo anti-CD22 con un enlazador, SPP (N-succinimidil 4-(2'-piridilditio) pentanoato, que ya puede estar unido a la molécula del fármaco o puede reaccionar posteriormente con una molécula del fármaco tal como un maitansinoide Por ejemplo, un anticuerpo se modifica mediante la reacción con SPP, seguida de la conjugación con f, tal como se describe en Wang, L. y col., *Protein Science* 14:2436-2446 (2005), cuyo

contenido se incorpora íntegramente en la presente memoria descriptiva a modo de referencia. Los residuos de lisina en un anticuerpo anti-CD22 también pueden reaccionar con un enlazador, SMCC (Pierce Chemical Co.), a pH 7-9 de manera que el N-hidroxisuccinimida amino reactivo (éster de NHS) de SMCC forme una unión amida estable con el anticuerpo. El grupo melaimido de reactivos sulfidrilos de SMCC reacciona con un grupo sulfidrilo de DM1 a pH 6.5-7.5 (véase Pierce Chemical Co., piercenet.com) para formar el ADC. Los residuos de lisina o cisteína reaccionan con un enlazador a fármacos para producir ADC que comprendan concentraciones de fármacos promedio de aproximadamente 1 y 8 moléculas de enlazadores a fármacos por anticuerpo, alternativamente de 1 a 6, 1 a 4, 1 a 3 ó 1 a 2 moléculas de enlazadores a fármacos por anticuerpo.

Los ACD anti-CD22 (RFB4)-SMCC-DM1 y anti-GP120-SMCC-DM1 se prepararon según este método, en el que RFB4-smcc-DM1 se preparó con concentraciones de fármacos bajas (1,95), medias (3,7) y altas (6,75). El anti-GP120-smcc-DM1 se preparó con una concentración de fármacos alta (6,1). Estos ACD demostraron ser eficaces *in vivo*, tal como se muestra a continuación en el ejemplo 9 y la Tabla 9 en la presente memoria descriptiva.

Ejemplo 9: Eficacia de los conjugados de anticuerpos anti-CD22 con fármacos

Estudios *in vitro* de los determinantes de la eficacia

Se delimitaron los determinantes de la eficacia de los ADC (o TDC) anti-CD22 en una línea celular de linfoma. Se conoce que el CD22 expresado en la superficie de células B se internaliza después de la unión de su(s) ligando(s) o anticuerpos (Sato, S. y col., *Immunity* 5:551-562 (1996)). Para determinar si y cómo el nivel de la expresión del CD22 en la superficie de las células B o su internalización afecta la eficacia, se realizaron los siguientes estudios *in vitro*.

Expresión en la superficie del CD22 humano en diversas líneas celulares de linfomas. Diecinueve líneas celulares de linformas que expresan diversas cantidades de CD22 en su superficie se cultivaron y recogieron en la fase exponencial del crecimiento. Las células se resuspendieron en un tampón de lavado FACS (PBS; 0,5% de albúmina de suero bobino; 0,1% de ázida sódica) que contiene 100 µg/ml de cada IgG de ratón normal e IgG human normal y que se hayan conservado en hielo. Aproximadamente 1 x 10^6 células/100 µl se tiñeron con APC anti-CD22 humano (mIgG1, clon RFB4, Southern Biotech #9361-11) o isotipo APC de IgG1 murina (BD Pharmingen #555751) durante 30 minutos en hielo. Las células muertas se tiñeron con 7-AAD (BD Pharmingen #559925). Los datos se obtuvieron en el citómetro de flujo BD FacsCalibur™ y se analizaron con el software FlowJo™. La determinación de IC50 para hu10F4v3-SMCC-DM1 o cada uno de los fármacos (DM1, MMAF o MMAE) se determinaron mediante cultivo de

células de linfoma, tal como se describió anteriormente, y recogida de las células cultivadas en la fase exponencial y sembrado de 5000 células en 90 µl de medio de cultivo por pocillo en una placa de 96 pocillos. El ADC y los fármacos se diluyeron en serie dentro del rango de detección (comenzando a 300 µg/ml para el ADC o 90 nM para los fármacos y diluyendo hasta lograr esencialmente el objetivo de cero ensayos). Se añadieron alícuotas de 10 µl de ADC diluido o de fármacos para replicar los pocillos que contienen células y se incubaron durante 3 días a 37 °C. Para cada pocillo, se añadió 100 µl de CellTiter Glo™ y se incubó durante 30 minutos. Se detectó quimioluminiscencia y los datos se analizaron utilizando el software Prism™. Los resultados se muestran en la Figura 6A, en la que los altos niveles de CD22 en superficie se correlacionan con los bajos niveles de IC50 (mayor eficacia) de hu10F4v3-SMCC-DM1. La Figura 6C indica que existe una correlación más fuerte entre la sensibilidad intrínseca de las células a los fármacos y la IC50 del ADC.

La internalización de hu10F4v3-SMCC-DM1 se determinó mediante el ensayo FACS. De manera resumida, las células de linfomas se tiñeron mediante técnicas estándar FACS con CD22-FITC (RFB4) en presencia de hu10F4v3-SMCC-DM1 y se incubaron en hielo entre 20 y 30 minutos. Para determinar los niveles de CD22 en la superficie de la célula después de la tinción inicial, las células se lavaron en un medio frío de RPMI con FBS al 10% y se añadió 200 µl de RPMI precalentado con FBS al 10% e incubó a 37 °C durante 15 minutos. Se añadió 80 µl de tampón de tinción y 20µl de suero de ratón normal inactivado por calor (HI NMS), a lo que prosiguió la inbucación en hielo durante 15 minutos. Se añadió Alexa-647 anti-DM1, se incubó en hielo entre 20 y 30 minutos y las células se lavaron y fjaron con 200 µl de paraformaldehído con PBS al 1% antes de realizar el análisis FACS. Para determinar la tinción interna y superficial del CD22 después de la tinción inicial, las células se lavaron con PRMI frío con FBS al 10%, se añadió RPMI precalentado con FBS al 10% y las células se incubaron durante 15 minutos a 37 °C. Posteriormente, las células se lavaron mediante la técnica FACS y se fijaron con el reactivo de fijación A (Dako™ #k2311) a temperatura ambiente durante 15 minutos; posteriormente, se repitió el paso con el reactivo de fijación B (Dako™). Se añadieron el tampón de tinción y el HI NMS, y la mezcla celular se incubó en hielo durante 15 minutos. Se añadió el reactivo de fijación B, a lo cual se agregó Alexa-647 anti-DM1 y se incubó a temperatura ambiente entre 20 y 30 minutos. Las células se lavaron mediante técnica FACS y se fijaron en paraformaldehído con PBS al 1%. El análisis FACS se realizó en cada mezcla celular (superficie, posinternalización superficial y tinción interna) utilizando un citómetro de flujo BD FacsCalibur™ y el software FlowJo™. Los resultados se muestran en la Figura 6B en la que grandes cantidades de DM1 internalizado se correlacionan con los bajos niveles de IC50 (alta eficacia) y en la Figura 6D en la que el DM1 internalizado se visualiza mediante microscopia de fluorescencia.

Estudios de eficacia *in vivo*

Se empleó el siguiente protocolo para determinar la eficacia de los anticuerpos anti-CD22 monoclonales no conjugados o conjugados con toxinas para poder reducir el volumen tumoral *in vivo*.

Cada ratón SCID se inoculó subcutáneamente en el flanco con 2 x 10^7 de línea celular de linfomas de células B humanas. Las líneas celulares humanas incluyeron las líneas celulares de linfoma de Burkitt, células Daudi, Ramos y Raji (disponibles en el American Type Culture Collection, Manassas, VA, EE.UU) y otras líneas de células B, incluyendo las células U-698-M y Su-DHL-4 (disponibles en DSMZ, Braunschweig, Alemania; las células Su-DHL-4 se tranfectaron con el gen indicador de luciferasa), células DoHH2 (Kluin-Neilemans, H.C. (1991), mencionado anteriormente) y Granta-519 (células de linfomas de células del manto, Jadayel, D.M. y col., *Leukemia* 11(1):64-72 (1997)) y células BJAB-luc (línea celular linfoblastoide de linfocitos humanos que expresa el gen indicador de luciferasa). Cuando los tumores alcanzaron un volumen tumoral medio de entre 100 y 200 mm3, los ratones se dividieron en grupos y se trataron en el día 0 con inyecciones intravenosas con anticuerpos conjugados con toxina o anticuerpos no conjugados, tal como se muestra en las siguientes Tablas 4 a la 16.

Los conjugados anti-CD22 con el fármaco maitansina reducen el volumen tumoral de las células B

Sesenta y cinco ratones SCID se inyectaron subcutáneamente con 2x10^7 de células BJAB-luc en un volumen de 0,2 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Cuando el tamaño del tumor medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en cuatro grupos de 9 ratones cada uno y se les administró un único tratamiento I.V. (a través de la vena de la cola) del anticuerpo anti-CD22 o del anticuerpo de control indicado en la siguiente Tabla 4.

Tabla 4

Reducción del volumen tumoral *in vivo*
Administración del anticuerpo

Anticuerpo administrado	TI	PR	CR	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)	Proporción del fármaco (Fracciones de fármaco/Ab)
anti-Her2-smcc-DM1	9/9	0	0	4.2	200	3.2
mu10F4-smcc-DM1	9/9	2	0	3.0	200	4.6
hu10F4v2-smcc-DM1	9/9	0	0	3.4	200	4.0
hu10F4v1	9/9	0	0	3.4	---	---

"TI": incidencia tumoral en el último momento de cada grupo; el numerador hace referencia a la cantidad de animales con tumores y el denominador hace referencia a la cantidad total de animales con tumores. "PR" hace referencia a la cantidad de animales con tumores que han disminuido entre el 50 y 99% de su volumen inicial. "CR" hace referencia a la cantidad de animales que lograron una remisión completa.

El volumen tumoral medio se monitorizó en cada grupo de tratamiento durante 32 días después de la inyección del anticuerpo. Las mediciones tumorales se realizaron con calibradores. La eficacia de los anticuerpos anti-CD22 conjugados con toxinas se determinó a partir de la comparación entre los anticuerpos de control y los anticuerpos no conjugados. Los resultados se muestran en la Figura 7A. Los anticuerpos monoclonales 10F4v1-smcc-DM1 humanizados y murinos ralentizaron considerablemente el crecimiento tumoral con respecto al anticuerpo anti-CD22 no conjugado y al anticuerpo de control no específico.

Utilizando el mismo protocolo anterior, se realizó un ensayo comparando el 10F4v2 humanizado conjugado con toxinas con el anticuerpo murino conjugado con toxinas y el anticuerpo humanizado desnudo, tal como se indica en la siguiente Tabla 5.

Tabla 5

Reducción del volumen tumoral *in vivo*
Administración del anticuerpo

Anticuerpo administrado	TI	PR	CR	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)	Proporción del fármaco (Fracciones de fármaco/Ab)
anti-Her2-smcc-DM1	9/9	0	0	4.2	200	3.2
mu10F4-smcc-DM1	7/9	1	2	4.7	200	2.9
hu10F4v2-smcc-DM1	8/9	1	1	4.5	200	3.0
hu10F4v2	9/9	0	0	4.5	---	---

"TI": incidencia tumoral en el último momento de cada grupo; el numerador hace referencia a la cantidad de animales con tumores en el grupo de estudio y el denominador hace referencia a la cantidad total de animales con tumores. "PR" hace referencia a la cantidad de animales con tumores que han disminuido entre el 50 y 99% de su volumen inicial. "CR" hace referencia a la cantidad de animales que lograron una remisión completa.

El volumen tumoral medio se monitorizó en cada grupo de tratamiento durante 32 días después de la inyección del anticuerpo. Las mediciones tumorales se realizaron con calibradores. La eficacia de los anticuerpos anti-CD22 conjugados con toxinas se determinó a partir de la comparación entre los anticuerpos de control y los anticuerpos no conjugados. Los resultados se muestran en la Figura 7B. Los anticuerpos monoclonales 10F4v2-smcc-DM1 humanizados y 10F4-smcc-DM1 murinos ralentizaron

considerablemente el crecimiento tumoral con respecto al anticuerpo anti-CD22 no conjugado y al anticuerpo de control no específico.

Un anticuerpo anti-CD22 se conjugó con DM1 mediante el enlazador spp o el enlazador smcc según los métodos de conjugación desvelados en la presente memoria descriptiva. El anticuerpo anti-CD22 desnudo se utilizó como control positivo y los conjugados de toxinas, anti-HER2-spp-DM1 y anti-HER2-smcc-DM1, se utilizaron como controles negativos. Ochenta ratones SCID se inyectaron subcutáneamente con 2x10^7 de células BJAB-luc en un volumen de 0,2 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en seis grupos de 10 ratones cada uno y se colocaron inyecciones intravenosas de anticuerpos de prueba o de control. Se repitieron las dosis una vez por semana para una cantidad total de tres dosis. Véase Tabla 6.

Tabla 6
Reducción del volumen tumoral *in vivo*
Administración del anticuerpo

Anticuerpo administrado	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)
anti-Her2-spp-DM1*	4	214
anti-Her2-smcc-DM1**	6.9	405
anti-CD22-spp-DM1*	5	214
anti-CD22-spp-DM1	2.5	107
anti-CD22-smcc-DM1**	10	405
Anti-CD22 desnudo	10	---

* Concentración de fármaco compatible
** Concentración de fármaco compatible

El volumen tumoral medio se monitorizó dos veces por semana durante 3 semanas y, posteriormente, una vez por semana desde ese momento durante un total de 8 semanas. Los cambios del volumen tumoral producidos durante el transcurso del tiempo (Figura 7C) muestran que el anti-CD22-spp-DM1 administrado a 214 y 107 µg/m^2 DM1 y que el anti-CD22-smcc-DM1 administrado a 405 µg/m^2 exhibieron actividad antitumoral sólida y comparable en tumores xenotransplantados de células BJAB-luc. Todos los grupos de ADC anti-CD22 demostraron respuestas completas.

Los anticuerpos anti-CD22, RFB4, 5E8 y 7A2 se conjugaron con DM1 mediante el enlazador smcc según los métodos de conjugación desvelados en la presente memoria descriptiva. Se utilizó anti-HER2-

smcc-DM1 conjugado con toxinas (en la presente memoria descriptiva, denominado indistintamente como HER-smcc-DM1 o HER2-smcc-DM1) como control negativo.

Se examinó la capacidad de estos anticuerpos de reducir el volumen tumoral en diferentes xenotransplantes en ratones SCID. Las líneas celulares de linfomas de células B humanas utilizadas para generar xenotransplantes de tumores en ratones fueron las células Ramos y BJAB-luc. Para cada xenotransplante, los ratones SCID se inyectaron subcutáneamente con 5x10^6 de células Ramos de linfoma de células B humanas en un volumen de 0,1 ml por ratón en el flanco (o 2x10^7 de células BJAB-luc en 0,2 ml). Las células se suspendieron en solución salina de Hanks. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. La concentración de fármaco DM1 se normalizó a 200 $\mu g/m^2$ para cada grupo para proporcionar la dosis de DM1 administrada. El volumen tumoral medio se monitorizó dos veces por semana durante 4 semanas. Los resultados se muestran a continuación en las Tablas 7 y 8 y se grafican en las Figuras 8A y 8B, respectivamente.

Tabla 7

Xenotransplante de células Ramos, reducción del volumen tumoral *in vivo*
Administración del anticuerpo

Anticuerpo administrado	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)
Anti-HER2-smcc-DM1	4.2	200
Anti-CD22(7A2)-smcc-DM1	3.8	200
Anti-CD22(7A2)-smcc-DM1	3.8	200
Anti-CD22(RFB4)-smcc-DM1	3.2	200

Tabla 8

Xenotransplante de células BJAB-luc, reducción del volumen tumoral *in vivo*
Administración del anticuerpo

Anticuerpo administrado	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)	Proporción del fármaco (Fracciones de fármaco/Ab)
Anti-HER2-smcc-DM1	4.2	200	3.2

Anti-CD22(7A2)-smcc-DM1	3.8	200	3.6
Anti-CD22(5E8)-smcc-DM1	3.8	200	3.6
Anti-CD22(RFB4)-smcc-DM1	3.2	200	4.25

Estos resultados muestran que los anticuerpos anti-CD22-smcc-DM1 conjugados con fármacos reducen considerablemente el volumen tumoral de las células B en los xenotranplantes de células Ramos y BJAB-luc con respecto al anticuerpo de control o al anticuerpo anti-CD22 desnudo.

Se examinó el efecto de la concentración de fármaco del anticuerpo (cantidad promedio de moléculas de fármaco conjugadas por anticuerpo en una población de anticuerpos) en la capacidad de los conjugados de anticuerpos anti-CD22-smcc-DM1 con fármacos para reducir el volumen tumoral en xenotransplantes de células BJAB-luc en ratones SCID. Ciento cuarenta ratones SCID se inyectaron subcutáneamente con 2x10^7 de células BJAB-luc en un volumen de 0,2 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. Se administraron las poblaciones de anti-CD22(RFB4)-smcc-DM1 con concentraciones altas, medias o bajas de fármaco (concentraciones de fármaco promedio de 1,95; 3,7 ó 6,75 conjugadas con moléculas de DM1 por anticuerpo, respectivamente) como anticuerpos de prueba. Los anticuerpos RFB4 desnudos y anti-GP120-smcc-DM1 (concentración de fármaco alta) fueron los controles. Las dosis de los conjugados de anticuerpos con fármacos (prueba y control) se normalizaron a una dosis de nivel de proteína de 5mg/kg. La unión de un conjugado enlazador a los anticuerpos se realizó a través de los residuos de lisina. Véase Tabla 9.

Tabla 9

Xenotransplante de células BJAB-luc, reducción del volumen tumoral *in vivo*

Administración de anti-CD22(RFB4)-smcc-DM1

Anticuerpo administrado	Dosis de Ab (mg/kg)	Dosis de DM1 ($\mu g/m^2$)	Proporción del fármaco (Fracciones de fármaco/ Ab)
Anti-CD22(RFB4) (anticuerpo desnudo)	10	--	--
anti-CD22(RFB4)-smcc-DM1 (bajo)	5	144	1.95
anti-CD22(RFB4) -smcc-DM1 (medio)	5	273	3.7
anti-CD22(RFB4)-smcc-	5	497	6.75

DM1 (alta)			
anti-GP120-smcc-DM1 (alta)	5	449	6.1

Cuando se administró a un nivel de proteína compatible (5 mg/kg), el anti-CD22(RFB4)-smcc-DM1 cargado con una concentración de fármaco alta (6,75 moléculas de DM1 por molécula de anticuerpo) redujo levemente el volumen tumoral más que el conjugado de anticuerpo con fármacos con una concentración media de 3,7, mientra que los efectos del conjugado de anticuerpo con fármacos con una concentración de fármacos baja no fue diferente a los del anticuerpo desnudo o conjugado de control. Los resultados se grafican en la Figura 9.

Los conjugados anti-CD22 con el fármaco auristatina reducen el volumen tumoral de las células B

Se examinó el efecto de los conjugados anti-CD22 con fármaco auristatina MMAF en el volumen tumoral en xenotransplantes de ratones. El anticuerpo anti-CD22(RFB4) y el anticuerpo de control anti-GP120 se conjugaron con MMAF a través de un enlazador MC-vcPAB o un enlazador MC según los métodos desvelados en la presente memoria descriptiva. Se inyectaron subcutáneamente ratones SCID con 5x10^6 de células Ramos en un volumen de 0,2 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. La dosis, la concentración del fármaco (proporción de fármaco) y la dosis de anticuerpo administrada a los ratones se muestra en la Tabla 10.

Tabla 10
Xenotransplante de células Ramos, reducción del volumen tumoral *in vivo*
Administración del conjugado de anti-CD22(RFB4) con MMAF

Anticuerpo administrado	Dosis de MMAF ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fracciones de fármaco/Ab)
anti-CD22(RFB4)-MCvcPAB-MMAF	405	6.6	4.2
anti-CD22(RFB4)-MC-MMAF	405	6.9	4.0
anti-GP120-MCvcPAB-MMAF	405	5.8	4.8
anti-GP120-MC-MMAF	405	5.9	4.7

El anti-CD22-MC-MMAF exhibió una actividad comparable al anti-CD22-MC-vc-PAB-MMAF en los xenotransplantes de las células Ramos RA1. Los resultados se grafican en la Figura 10.

Se examinó el efecto de los conjugados anti-CD22 con fármaco auristatina MMAE y DM1 en el volumen tumoral en xenotransplantes de ratones. El anticuerpo anti-CD22(RFB4) y el anticuerpo de control anti-GP120 se conjugaron con MMAE a través de un enlazador MC-vcPAB o un enlazador MC, o con DM1 a través de un enlazador smcc según los métodos desvelados en la presente memoria descriptiva. Se inyectaron subcutáneamente ratones SCID con 5x10^6 de células Ramos en un volumen de 0,1 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Se administró PBS como control. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. La dosis, la concentración del fármaco (proporción de fármaco) y la dosis de anticuerpo administrada a los ratones se muestra en la Tabla 11.

Tabla 11
Xenotransplante de células Ramos, reducción del volumen tumoral *in vivo*
Administración del conjugado de DM1 anti-CD22(RFB4) con MMAE y DM1

Anticuerpo administrado	Dosis de MMAE o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fracciones de fármaco/Ab)
Anti-GP120-smcc-DM1	405	6.7	4.1
Anti-CD22(RFB4)-smcc-DM1	405	6.5	4.25
Anti-GP120-MCvcPAB-MMAE	405	6.0	4.7
antiCD22(RFB4)-MCvcPAB-MMAE	405	6.3	4.5
PBS	--	--	--

El anti-CD22-MCvcPAB-MMAE exhibió actividad antitumoral potente en los xenotransplantes de células Ramos RA1. El anti-CD22-MCvcPAB-MMAE exhibió actividad superior en comparación con el antiCD22-smcc-DM1. El ADC de control, anti-GP120-MCvcPAB-MMAE, no exhibió actividad considerable. Los resultados se grafican en la Figura 11.

Se examinó el efecto de los conjugados anti-CD22 con fármaco auristatina MMAF y DM1 en el volumen tumoral en xenotransplantes de ratones. Se administraron anti-CD22 hu10F4v2-MC-MMAF, hu10F4v2-smcc-DM1 y tio-10F4v1-MC-MMAF, y se compararon para determinar el efecto en el volumen tumoral. Los anticuerpos de control fueron anti-Her2-MC-MMAF y anti-Her2-smcc-DM1. Se inyectaron ratones SCID subcutáneamente con 2x10^7 de células BJAB-luc en un volumen de 0,2 ml por ratón en el flanco. Las células se suspendieron en solución salina de Hanks. Cuando el tamaño tumoral medio alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. "Tio" hace referencia a thioMab, tal como desveló en la presente memoria descriptiva, en el que la fracción del

enlazador a fármaco se conjuga con el anticuerpo a través de un sitio del anticuerpo diseñado con cisteína. La dosis, la concentración del fármaco (proporción de fármaco) y la dosis de anticuerpo administrada a los ratones se muestra en la Tabla 12.

Tabla 12
Xenotransplante de células BJAB-luc, reducción del volumen tumoral *in vivo*
Administración del conjugado de Hu10F4 con MMAF y DM1

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fracciones de fármaco/Ab)
Anti-Her2-MC-MMAF	100	1.1	6.3
Hu10F4v2-MC-MMAF	100	2.0	3.4
Hu10F4v2-MC-MMAF	50	1.0	3.4
Tio-hu10F4v1-MC-MMAF	100	4.6	1.5
Tio-hu10F4v1-MC-MMAF	50	2.3	1.5
Anti-Her2-smcc-DM1	200	4.2	3.2
Hu10F4v2-smcc-DM1	200	4.5	3.0
Hu10F4v2-smcc-DM1	100	2.3	3.0

Los Hu10F4v2 ADC exhibieron una actividad antitumoral potente en los xenotransplantes de células BJAB-luc. Los resultados se grafican en la Figura 12.

Utilizando los procedimientos que se desvelaron en los experimentos anteriores, se examinó la eficacia de hu10F4v3 –smcc-DM1 y –MC-MMAF ADC en diferentes xenotransplantes con diferentes dosis. Los xenotransplantes de SuDHL4-luc, DoHH2 y -519 se prepararon tal como se desveló anteriormente en la presente memoria descriptiva. Cuando el tamaño tumoral alcanzó 100 y 200 mm^3, los ratones se agruparon aleatoriamente en grupos de entre 8 y 10 ratones cada uno, y a cada ratón se le colocó una inyección intravenosa de anticuerpos de prueba o de control. Se les administraron a los ratones la dosis y la concentración de fármaco (proporción de fármaco), y la dosis de anticuerpo, tal como se muestra en las Tablas 136A-13C y los resultados se muestran en las Figuras 13A-13C.

Tabla 13A
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Hu10F4v3 con MMAF y DM1
en xenotransplantes de células SuDHL-4-luc

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fármaco/Ab)
Anti-Her2-smcc-DM1	600	11.9	3.3
Hu10F4v3-smcc-DM1	600	13.6	2.9
Hu10F4v3-smcc-DM1	300	6.8	2.9
Anti-Her2-MC-MMAF	600	9.9	4.0
Hu10F4v3-MC-	600	13.3	3.0

MMAF			
Hu10F4v3-MC-MMAF	300	6.6	3.0

Tabla 13B
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Hu10F4v3 con MMAF y DM1
en xenotransplantes de células DoHH2

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fármaco/Ab)
Anti-Her2-smcc-DM1	600	11.9	3.3
Hu10F4v3-smcc-DM1	600	11.8	3.35
Hu10F4v3-smcc-DM1	300	5.9	3.35
Anti-Her2-MC-MMAF	600	9.9	4.0
Hu10F4v3-MC-MMAF	600	13.1	3.04
Hu10F4v3-MC-MMAF	300	6.6	3.04
hu10F4v3 desnudo	--	13.1	--

Tabla 13C
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Hu10F4v3 con MMAF y DM1
en xenotransplantes de células Granta-519

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fármaco/Ab)
Anti-Her2-smcc-DM1	300	5.9	3.3
Hu10F4v3-smcc-DM1	300	5.9	3.35
Hu10F4v3-smcc-DM1	150	2.9	3.35
Anti-Her2-MC-MMAF	300	4.9	4.0
Hu10F4v3-MC-MMAF	300	6.6	3.04
Hu10F4v3-MC-MMAF	150	3.3	3.04
hu10F4v3 desnudo	--	6.6	--

El anti-CD22 hu10F4v3 –smcc-DM1 y -MC-MMAF ADCs exhibieron una reducción tumoral potente en todos los modelos de xenotransplantes evaluados.

Ejemplo 10: Preparación de anticuerpos anti-CD22 diseñados con cisteína

La preparación de anticuerpos anti-CD22 diseñados con cisteína se realizó tal como se desveló en la presente memoria descriptiva. El ADN que codifica el anticuerpo 10F4v3, con las mismas secuencias

de regiones constantes y variables que 10F4v2 (cadena ligera, identificador de secuencia n.º: 87 y cadena pesada, identificador de secuencia n.º: 88, Figura 5B) se mutagenizó mediante los métodos desvelados en la presente memoria descriptiva para modificar la cadena ligera, la cadena pesada o la región FC de la cadena pesada. El ADN que codifica la cadena ligera se mutagenizó para sustituir la cisteína por valina en la posición 205 según la numeración de Kabat en la cadena ligera (posición secuencial 210), tal como se muestra en la Figura 17A (cadena ligera, identificador de secuencia n.º: 91 del thiomab 10F4v3 del anticuerpo humanizado). El ADN que codifica la cadena pesada se mutagenizó para sustituir la cisteína por alanina en la posición 118 según la numeración EU en la cadena pesada (posición secuencial 121), tal como se muestra en la Figura 17B (cadena pesada, identificador de secuencia n.º: 92 del thiomab 10F4v3 del anticuerpo humanizado). La región Fc se mutagenizó para sustituir la cisteína por serina en la posición 400 según la numeración EU en la región Fc de la cadena pesada (posición secuencial 403), tal como se muestra en la Figura 17C (cadena pesada, identificador de secuencia n.º: 93).

Preparación de anticuerpos anti-CD22 diseñados con cisteína para conjugarlos mediante reducción y reoxidación

Los anticuerpos monoclonales anti-CD22 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/Ab 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 11: Preparación de anticuerpos anti-CD22 diseñados con cisteína y conjugados con fármacos por medio de conjugación de anticuerpos anti-CD22 diseñados con cisteína y enlazadores a fármacos intermedios

Después de los procedimientos de reducción y reoxidación del Ejemplo 10, el anticuerpo anti-CD22 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-CD22 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.

La preparación de hu 10F4v3 HC(A118C) thiomab-BMPEO-DM1 se realizó de la siguiente manera. El anticuerpo sin cisteína en hu 10F4v3 HC(A118C) thiomab fue modificado por el reactivo de bis-maleimida BM(PEO)4 (Pierce Chemical), lo cual dejo un grupo malaimido no reaccionado en la superficie del anticuerpo. Esto se logró mediante la disolución de BM(PEO)4 en una mezcla de 50% de etanol/agua a una concentración de 10 mM y añadiendo un exceso molar de BM(PEO)4 de diez veces a una solución que contiene hu4D5Fabv8-(V110C) ThioFab en una solución salina fosfatada de aproximadamente 1,6 mg/ml (10 micromolares) y permitiendo que reaccione durante 1 hora. El exceso de BM(PEO)4 se eliminó por filtración en gel (columna HiTrap, Pharmacia) en 30 mM de citrato, pH 6 con 150 mM de tampón de NaCl. Aproximadamente un exceso molar de DM1 de 10 veces disuelto en dimetilacetamida (DMA) se agregó al hu4D5Fabv8-(V110C) ThioFab-BMPEO intermedio. Asimismo, puede emplearse dimetilformamida (DMF) para disolver el reactivo de fracciones de fármaco. Se permitió que la mezcla de reacción reaccione durante la noche antes de la filtración en gel o diálisis en PBS para eliminar el fármaco no reaccionado. La filtración en gel en las columnas S200 en PBS se utilizó para eliminar los agregados de alto peso molecular y para suministrar hu 10F4v3 HC(A118C) thiomab-BMPEO-DM1 purificado.

A partir de los mismos protocolos, se prepararon anticuerpos de control HC (A118C) MAb-MC-MMAF, HC ThioMAb-MC-MMAF, HCThioMAb-MCvcPAB-MMAE y HC ThioMab-BMPEO-DM1.

Siguiendo los procedimientos anteriores, se prepararon y probaron los siguientes anticuerpos anti-CD22 conjugados con fármacos y diseñados con cisteína:

tio hu tio-HC-10F4v3-MC-MMAF por conjugación de A118C tio hu 10F4v3 y MC-MMAF;

tio hu tio-HC-10F4v3-MC-val-cit-PAB-MMAE por conjugación de A118C tio hu 10F4v3 y MC-val-cit-PAB-MMAE;

tio hu HC-10F4v3-bmpeo-DM1 por conjugación de A118C tio hu HC-10F4v3 y bmpeo-DM1;

tio hu LC-10F4v3-MC-val-cit-PAB-MMAE por conjugación de V205C tio hu LC-10F4v3 y MC-val-cit-PAB-MMAE; y

tio hu Fc-10F4v3-MC-val-cit-PAB-MMAE por conjugación de S400C tio hu Fc-10F4v3 y MC-val-cit-PAB-MMAE.

Ejemplo 12: Caracterización de la afinidad de unión de los conjugados de ThioMAb diseñados con cisteína con fármacos al antígeno de superficie celular

La afinidad de unión de los conjugados de tio hu 10F4v3 con fármacos al CD22 expresada en las células BJAB-lucs se determinó mediante análisis FACS. De manera resumida, entran en contacto aproximadamente 1x10^6 células en 100 µl con diferentes cantidades de alguno de los siguientes conjugados de ThioMAb anti-CD22 con fármacos: tio hu LC(V205C) 10F4v3-MCvcPAB-MMAE, tio hu Fc(S400C) 10F4v3-MCvcPAB-MMAE, tio hu HC(A118C) 10F4v3-MCvcPAB-MMAE, tio hu HC(A118C) 10F4v3-MC-MMAF o tio hu HC(A118C) 10F4v3-BMPEO-DM1 (véanse las Figuras 18A-18E, respectivamente). El anticuerpo anti-CD22 unido a la superficie de la célula se detectó utilizando anti-huFc de cabra biotinilado más estreptavidina-PE. Los gráficos de las Figuras 18A-18E indican que la unión a antígeno fue aproximadamente la misma para todos los conjugados de thiomab con fármacos probados.

Ejemplo 13: Ensayo para reducir el volumen tumoral *in vivo* mediante conjugados de ThioMab anti-CD22 con fármacos

La capacidad de los conjugados de thiomab con fármacos preparados según el Ejemplo 11 para reducir el volumen tumoral de las células B en modelos de xenotransplantes se evaluó según el procedimiento desvelado en el Ejemplo 9 de la presente memoria descriptiva. A los ratones SCID con tumores de xenotransplantes de células Granta-519, el día 0 se le administraron conjugados de thiomab anti-CD22 humanizado 10F4v3 con fármacos y conjugados de control, tal como se muestra en la siguiente Tabla 14. El thiomab HC(A118C) de control fue un anticuerpo anti-HER2 4D5.

Tabla 14
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Tio Hu10F4v3 con MMAE y MMAF
en xenotransplantes de células Granta-519

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco

			(Fármaco/ Ab)
Tio HC(A118C)-MC-MMAF de control	100	3.99	1.65
Tio HC(A118C)-MCvcPAB-MMAE de control	100	4.33	1.55
Tio 10F4v3-HC(A118C)-MC-MMAF	100	3.41	1.95
Tio 10F4v3-LC(V205C)-MCvcPAB-MMAE	100	4.23	1.6
Tio 10F4v3-HC(A118C)-MCvcPAB-MMAE	100	3.76	1.8
Tio 10F4v3-Fc(S400C)-MCvcPAB-MMAE	100	4.23	1.6

Los resultados de este experimento se muestran en la Figura 19. La administración de los conjugados de thiomab tio 10F4v3-LC-(V205C)-MCvcPAB-MMAE y tio 10F4v3-HC(A118C)-MCvcPAB-MMAE con fármacos en las dosis que se muestran en la Tabla 14 redujeron el volumen tumoral medio durante la realización del estudio.

Se evaluaron conjugados de thiomab con fármacos adicionales en los xenotransplantes de células Granta-519 en ratones CB17 SCID utilizando el mismo protocolo, aunque se probaron diferentes dosis del fármaco. El anticuerpo de control o thiomab de control fue un anticuerpo anti-HER2 4D5 o thiomab HC(A118C). Los resultados se muestran en la siguiente Tabla 15.

Tabla 15
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Tio Hu10F4v3 con MMAE, MMAF y DM1
en xenotransplantes de células Granta-519

Anticuerpo administrado	Dosis de MMAF o DM1 ($\mu g/m^2$)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fármaco/ Ab)
10F4v3-MC-MMAF	150	3.2	3.1
Tio HC(A118C)-BMPEO-DM1 de control	300	10.3	1.9
Tio 10F4v3-HC(A118C)-BMPEO-DM1	150	5.2	1.9
Tio 10F4v3-HC(A118C)-BMPEO-DM1	300	10.4	1.9
Tio HC(A118C)-MCvcPAB-MMAE de control	150	6.5	1.55
Tio 10F4v3-HC(A118C)-MCvcPAB-MMAE	150	5.3	1.9
Tio 10F4v3-HC(A118C)-MCvcPAB-MMAE	75	2.7	1.9
Tio HC(A118C)-MC-MMAF de control	150	5.2	1.9
Tio 10F4v3-HC(A118C)-MC-MMAF	150	5.1	1.95
Tio 10F4v3-HC(A118C)-MC-MMAF	75	2.6	1.95

Los resultados de este experimento se muestran en la Figura 20A. La administración del conjugado del thiomab tio 10F4v3-HC(A118C)-MCvcPAB-MMAE con fármaco a 150 y 75 μg/m^2 redujo el volumen tumoral medio durante la realización del estudio. En el mismo estudio, el cambio porcentual del peso corporal durante los primeros siete días se determinó en cada grupo de dosis. Los resultados graficados en la Figura 20B indican que la administración de estos conjugados de thiomab con fármacos no ocasionaron pérdida de peso durante este período de tiempo.

En un estudio similar, utilizando el mismo protocolo del estudio de xenotransplante tal como se desveló en los ejemplos anteriores y variando las dosis y TDC administrados, se analizó la eficacia de los TDC en xenotransplantes de DOHH2 con linfomas foliculares en ratones CB17 SCID. Los TDC y las dosis se muestran en la siguiente Tabla 16.

Tabla 16
Reducción del volumen tumoral *in vivo*
Administración del conjugado de Tio Hu10F4v3 con MMAE, MMAF y DM1
en xenotransplantes de células DOHH2

Anticuerpo administrado	Dosis de MMAF o DM1 (μg/m^2)	Dosis de Ab (mg/kg)	Proporción del fármaco (Fármaco/ Ab)
10F4v3-MC-MMAF	300	6.4	3.1
Tio HC(A118C)-BMPEO-DM1 de control	600	21.9	1.79
Tio 10F4v3-HC(A118C)-BMPEO-DM1	600	20.8	1.9
Tio 10F4v3-HC(A118C)-BMPEO-DM1	300	10.4	1.9
Tio HC(A118C)-MCvcPAB-MMAE de control	600	26.0	1.55
Tio 10F4v3-HC(A118C)-MCvcPAB-MMAE	600	21.4	1.9
Tio 10F4v3-HC(A118C)-MCvcPAB-MMAE	300	10.7	1.9
Tio HC(A118C)-MC-MMAF de control	600	20.8	1.9
Tio 10F4v3-HC(A118C)-MC-MMAF	600	20.4	1.95
Tio 10F4v3-HC(A118C)-MC-MMAF	300	10.2	1.95

La Figura 20C es un gráfico que muestra los cambios en el volumen tumoral medio producidos durante el transcurso del tiempo en los xenotransplantes de DOHH2 con linfomas foliculares en ratones CB17 SCID tratados con los mismos A118C anti-CD22 TDC de cadena pesada pero con dosis más elevadas, tal como se muestra en la Tabla 16. El anti-CD22 10F4v3-HC(A118C)-MCvcPAB-MMAE TDC aparentemente fue el más eficaz de los agentes de prueba de este estudio. Sin embargo, en los niveles de

dosis más elevados en este experimento, se observó cierta eficacia en los controles anti-HER2-HC(A118C)-MCvcPAB-MMAE. Esta actividad posiblemente se atribuya a la liberación del fármaco del ADC en circulación. Los agentes de prueba anti-CD22 hu10F4-HC(A118C)-MC-MMAF y –BMPEO-DM1 exhibieron un grado intermedio de eficacia y, consecuentemente con la mayor estabilidad de estos enlazadores, los controles anti-HER2 sin unión demostraron poca actividad. La Figura 20D es un diagrama sobre el porcentaje de cambios de peso en los ratones utilizados en el estudio del xenotransplante DOHH2 que muestra que no se produjo ningún cambio de peso significativo durante los primeros 14 días del estudio.

Ejemplo 14: Seguridad de los conjugados de anti-CD22 con fármacos en ratas y monos cinomolgus

El anticuerpo anti-CD22 hu10F4 reacciona con el CD22 de mono cinomolgus con una afinidad equivalente a la del CD22 humano. El anticuerpo anti-CD22 hu10F4 no reacciona con el CD22 de rata. En consecuencia, la toxicidad y seguridad dependiente e independiente del elemento diana de los conjugados de anti-CD22 con fármacos se evaluaron en ratas y monos cinomolgus respectivamente.

Seguridad y toxicidad en ratas

Se realizaron dos estudios para evaluar la seguridad y toxicidad en ratas. En uno de los estudios, las ratas recibieron en el día 1 dosis intravenosas de conjugados de hu10F4v3-SMCC-DM1, -SPP-DM1, -MC-vc-PAB-MMAE o –MC-MMAF, en los que el fármaco se unió a través de un enlazador escindible (-vc- o –spp-) o no escindible (MC o SMCC (también denominado MCC)). El vehículo se administró como el control. El día 5, se extrajeron las muestras de sangre para realizar análisis farmacocinéticos; también se recogieron el día 12 (durante la necropsia). Se registraron las observaciones clínicas y el peso corporal al menos tres veces por semana. Se monitorizó el AST (aspartato aminotransferasa) en suero como indicador de toxicidad. Los niveles de AST en suero incrementaron el día 5 en relación con el día 0 en ratas que recibieron dosis de 20 mg/kg de hu10F4v3-vcMMAE y hu10F4v3-SPP-DM1 con enlazadores escindibles (Figura 21A). Los niveles de neutrófilo se incrementaron el día 5 en relación con el día 0 en ratas que recibieron dosis de 20 mg/kg de hu10F4v3-MC-MMAF o hu10F4v3-MCC-DM1 con enlazadores no escindibles (Figura 21B). Los niveles de neutrófilo disminuyeron el día 5 en relación con el día 0 en ratas que recibieron dosis de hu10F4v3-vc-MMAE o hu10F4v3-SPP-DM1. El incremento del AST en suero y la disminución de los neutrófilos en ratas que recibieron dosis de ADC con enlazadores escindibles indican mayor toxicidad por parte de dichos ADC.

En el mismo estudio realizado con ratas, seis animales por grupo recibieron dosis de 20, 40 ó 60 mg/kg de hu10F4v3-MC-MMAF o hu10F4v3-SMCC-DM1 el día 1 y se monitorizaron durante doce días. En animales que recibieron dosis de hu10F4v3-MC-MMAF, no se registraron observaciones en los siguientes indicadores: disminución en el peso corporal, incrementos en las enzimas séricas del hígado, disminuciones en las plaquetas o disminuciones en los neutrófilos. En ratas que recibieron dosis de hu10F4v3-SMCC-DM1, las disminuciones reversibles del peso corporal y los incrementos reversibles en las enzimas séricas del hígado se observaron en los niveles de dosificación de 40 y 60 mg/kg, mientras que las disminuciones reversibles en los neutrófilos y las disminuciones transitorias en las plaquetas se observaron en la dosificación de 60 mg/kg.

Seguridad y toxicidad en monos cinomolgus

Para evaluar la seguridad y toxicidad de los ADC de anti-CD22 en un modelo de primates, se asignaron treinta monos cinomolgus a los siguientes grupos de tratamiento: control de vehículo (6 animales), hu10F4v3-SMCC-DM1 en dosis de 2, 4 y 6 mg/m^2 del fármaco (equivalente a 0, 10, 20 y 30 mg/kg de la dosis del anticuerpo, 4 animales por grupo de dosificación) y hu10F4v3-MC-MMAF en dosis de 2, 4 y 6 mg/m^2 (4 animales por grupo de dosificación). Los días 1 y 22, los animales recibieron dosis intravenosas. Se evaluaron para detectar cambios en el peso corporal, consumo de alimentos e índices patológicos. Se extrajeron las muestras de sangre y se analizaron para evaluar los efectos toxicológicos, farmacodinámicos y antifármacos del anticuerpo. La mitad de los animales en cada grupo se sacrificaron en los días 25 y 43, y se recolectaron muestras de tejido.

No se observaron cambios notables en el peso corporal en ningún grupo de ADC. Los niveles de las enzimas séricas del hígado AST (aspartato aminotransferasa), ALT (aminotransferasa) y GGT (gama-glutamil transpeptidasa) se analizaron según los métodos estándar bien conocidos en los sectores pertinentes. Los incrementos reversibles en las enzimas séricas del hígado se observaron en animales que recibieron dosis de 30 mg/kg de cualquier ADC, a pesar de que la ALT se incrementó en el grupo DM1 y que la AST y GGT se incrementaron en el grupo MMAF. La degeneración del nervio ciático fue entre mínima y leve en el grupo DM1 en 2 de 4 animales que recibieron dosis de 20 mg/kg y en 4 de 4 animales que recibieron dosis de 30 mg/kg. La degeneración del nervio ciático fue mínima en el grupo MMAF en 1 de 4 animales que recibieron dosis de 30 mg/kg. Se examinó microscópicamente tejido de diversos órganos. Dos de cuatro animales en el grupo de MMAF que recibieron dosis de 30 mg/kg sufrieron lesiones pulmonares de importancia desconocida, mientras que no se observaron lesiones en el grupo DM1.

La reducción de células B periféricas por la acción de los ADC de hu10F4v3-MC-MMAF y –SMCC-DM1 se determinó a partir de la medición de los niveles de células $CD20^+$ en sangre durante 43 días en monos cinomolgus que recibieron dosis los días 0 y 22. Durante el estudio, se analizó la sangre extraída periódicamente mediante análisis FACS utilizando un anticuerpo anti-CD20 marcado fluorescentemente. Los ADC de anti-CD22 MMAF y DM1 reducen las células B periféricas de los monos cinomolgus, tal como se muestra en la Figura 22A (grupo MMAF) y en la Figura 22B (grupo DM1). No se observaron efectos significativos de los ADC de MMAF o DM1 para otras poblaciones de linfocitos, tal como se muestra en las Figuras 23A y 23B, en las que se muestra que las células $CD4^+$ no se redujeron significativamente durante el mismo período de tiempo.

El Hu10F4v3-SMCC-DM1 redujo las células B del centro germinal en las muestras tonsilares de los monos cinomolgus con respecto al control, tal como se muestra en los fotomicrográficos de las Figuras 24A y 24B. Los centros germinales ejemplares aparecen encerrados con un círculo en la Figura 24A. La ablación completa de las células B del centro germinal se observó en el nivel de dosificación de 10 mg/kg, tal como se muestra en la Figura 24B. Se obtuvieron los mismos resultados administrando el ADC de hu10F4v3-MC-MMAF en las mismas condiciones.

El Hu10F4v3-MC-MMAF administrado a 10 mg/kg redujo las células B que se dividen de los centros germinales de los folículos linfoides de los monos cynomolgus. Véase el diagrama de la Figura 25A y los fotomicrográficos de tejido en las Figuras 25B y 25C. Los mismos resultados se obtuvieron cuando el ADC de hu10F4v3-SMCC-DM1 se probó en las mismas condiciones. Los centros germinales aparecen como regiones oscuras en la Figura 25B utilizando tinción Ki-67 y aparecen en la Figura 25D como áreas sin tinción rodeadas de regiones oscuras cuando se los tiñe con anti-IgD y se los marca de manera detectable. La pérdida de los centros germinales ocasionada por la reducción de las células B de los centros germinales por anti-10F4v3-MC-MMAF se muestra en las Figuras 25C y 25E. Por lo tanto, estos fármacos antimitóticos afectan las poblaciones de células B en proliferación.

En la American Type Culture Collection, PO Box 1549, Manassas, VA, 20108, Estados Unidos (ATCC) se ha depositado el siguiente hibridoma:

Líneas celulares	N.° de acceso de ATCC	Fecha de depósito
Hibridoma 10F4.4.1	PTA-7621	6 de mayo de 2006
Hibridoma 5E8.1.8	PTA-7620	6 de mayo de 2006

Estos depósitos se realizaron de conformidad con los términos del Tratado de Budapest sobre el Reconocimiento internacional del depósito de microorganismos a los fines del procedimiento en materia de

patentes y el Reglamento del mismo (Tratado de Budapest). Esto asegura el mantenimiento de un depósito viable durante 30 años a partir de la fecha de depósito. La ATCC pondrá a disposición estas líneas celulares según los términos del Tratado de Budapest y sujeto al acuerdo entre Genentech, Inc. y ATCC, que garantiza la disponibilidad permanente e ilimitada al público de las líneas celulares del depósito una vez concedida la patente de EE.UU. pertinente o una vez que se haga pública cualquier solicitud de patente de EE.UU. o extranjera, lo que ocurra primero, y asegura la disponibilidad de las líneas celulares a la persona que determine el Comisionado de Patentes y Marcas Registradas de EE.UU. para que tenga derecho a las mismas, en función del artículo 122 del Título 35 del Código de EE.UU. (35 USC § 122) y las normas del Comisionado de conformidad con lo anterior (incluyendo el artículo 1.14 del Título 37 del Código de Normas Federales (37 CFR § 1.14), con especial referencia a 886 OG 638).

El beneficiario de la presente solicitud ha aceptado que en caso de que las líneas celulares depositadas se pierdan o resulten destruidas cuando se cultiven en las condiciones adecuadas, serán inmediatamente sustituidas previa notificación con especímenes de la misma línea celular. La disponibilidad de las líneas celulares depositadas no debe interpretarse como una licencia para practicar la invención en contravención de los derechos otorgados por la autoridad de cualquier gobierno, de acuerdo con sus leyes de patentes.

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 que se une a CD22, donde el anticuerpo comprende (a) una HVR-L1 que contiene una secuencia de aminoácidos seleccionada de un identificador de secuencia n.º: 9, 10, 19-23, 32 y 33, y (b) al menos una, dos, tres, cuatro o cinco HVR seleccionadas entre:
   (1) una HVR-H1 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 2;
   (2) una HVR-H2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 4;
   (3) una HVR-H3 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 6;
   (4) una HVR-L2 que comprenda la secuencia de aminoácidos de identificador de secuencia n.º: 12; y
   (5) una HVR-L3 que comprenda una secuencia de aminoácidos de identificador de secuencia n.º: 14.
2. El anticuerpo de la reivindicación 1, que comprende una HVR-L1 que comprenda una secuencia de aminoácidos que se adapte a la secuencia de consenso de identificador de secuencia n.º: 10.
3. El anticuerpo de la reivindicación 2, que comprende, además, una HVR-H1 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 2 y una HVR-H2 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 4, y una HVR-H3 que contiene la secuencia de aminoácidos de identificador de secuencia n.º: 6.
4. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 9 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.
5. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 19 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.
6. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 20 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.
7. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 21 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de

identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.

8. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 22 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.

9. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 23 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.

10. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 32 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.

11. El anticuerpo de la reivindicación 1, donde la HVR-L1 comprende el identificador de secuencia n.º: 33 y el anticuerpo comprende, además, una HVR-H1 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 2, una HVR-H2 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 4 y una HVR-H3 que comprende una secuencia de aminoácidos de identificador de secuencia n.º: 6.

12. El anticuerpo de cualquiera de las reivindicaciones 1, que comprende, además, al menos una estructura seleccionada entre una estructura de consenso del subgrupo III de VH y una estructura de consenso del subgrupo I de VL.

13. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende un dominio variable de la cadena pesada con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 16.

14. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende un dominio variable de la cadena ligera con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 17.

15. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende un dominio variable de la cadena ligera con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 18.

16. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende un dominio variable de la cadena pesada que contiene uno, dos, tres o cuatro estructuras de secuencias de aminoácidos seleccionadas del identificador de secuencia n.º: 1, 3, 5 y 7.

17. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende un dominio variable de la cadena ligera que contiene uno, dos, tres o cuatro estructuras de secuencias de aminoácidos seleccionadas del identificador de secuencia n.º: 8, 11, 13 y 15.

18. El anticuerpo de la reivindicación 13, que comprende, además, un dominio variable de la cadena ligera con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 17.

19. El anticuerpo de la reivindicación 13, que comprende, además, un dominio variable de la cadena ligera con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 18.

20. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende una cadena pesada con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 88.

21. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende una cadena ligera con al menos un 90% de identificación de secuencia con respecto a la secuencia de aminoácidos seleccionada del identificador de secuencia n.º: 87.

22. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende una cadena pesada que contiene la secuencia de aminoácidos del identificador de secuencia n.º: 88, y una cadena ligera que contiene la secuencia de aminoácidos del identificador de secuencia n.º: 87.

23. Un anticuerpo que se une a CD22, donde el anticuerpo comprende un dominio variable de la cadena pesada con al menos un 90% de identificación de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º: 16.

24. El anticuerpo de la reivindicación 23, que comprende, además, un dominio variable de la cadena ligera con al menos un 90% de identificación de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º:17.

25. El anticuerpo de la reivindicación 23, que comprende, además, un dominio variable de la cadena ligera con al menos un 90% de identificación de la secuencia con respecto a la secuencia de aminoácidos de identificador de secuencia n.º:18.

26. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende uno, dos, tres, cuatro, cinco o seis HVR del anticuerpo que produce el hibridoma de ATCC, entrada n.º PTA-7621 (10F4.4.1).

27. Un anticuerpo que comprende uno, dos, tres, cuatro, cinco o seis HVR del anticuerpo producido por el hibridoma de ATCC, entrada n.º PTA-7620 (5E8.1.8).

28. El anticuerpo de la reivindicación 1, donde el anticuerpo es humanizado.
29. El anticuerpo de la reivindicación 23, donde el anticuerpo es humanizado.
30. El anticuerpo de la reivindicación 1, donde CD22 es un CD22 mamífero.
31. El anticuerpo de la reivindicación 30, donde el CD22 se selecciona del CD22 de un roedor y el CD22 de un primate.
32. El anticuerpo de la reivindicación 31, donde CD22 es un CD22 humano.
33. El anticuerpo de la reivindicación 23, donde CD22 es un CD22 mamífero.
34. El anticuerpo de la reivindicación 33, donde el CD22 se selecciona del CD22 de un roedor y el CD22 de un primate.
35. El anticuerpo de la reivindicación 34, donde CD22 es un CD22 humano.
36. Un polinucleótido que codifica un anticuerpo de la reivindicación 1.
37. Un polinucleótido que codifica un anticuerpo de la reivindicación 23.
38. Un vector que comprenda el polinucleótido de la reivindicación 36.
39. Un vector que comprenda el polinucleótido de la reivindicación 37.
40. Una célula huésped que comprende el vector de la reivindicación 38.
41. Una célula huésped que comprende el vector de la reivindicación 39.
42. La célula huésped de la reivindicación 40, donde la célula huésped es eucariótica.
43. La célula huésped de la reivindicación 42, donde la célula huésped es una célula CHO.
44. La célula huésped de la reivindicación 41, donde la célula huésped es eucariótica.
45. La célula huésped de la reivindicación 44, donde la célula huésped es una célula CHO.
46. Un método de fabricación de un anticuerpo anti-CD22, en el que el método comprende a) el cultivo de la célula huésped de la reivindicación 38 en condiciones aptas para la expresión del polinucleótido que codifica el anticuerpo, y b) el aislamiento del anticuerpo.
47. Un método de fabricación de un anticuerpo anti-CD22, en el que el método comprende a) el cultivo de la célula huésped de la reivindicación 39 en condiciones aptas para la expresión del polinucleótido que codifica el anticuerpo, y b) el aislamiento del anticuerpo.
48. El anticuerpo de la reivindicación 28, donde el CD22 se expresa en la superficie de una célula.
49. El anticuerpo de la reivindicación 48, en el que la célula es una célula B.
50. El anticuerpo de la reivindicación 29, donde el CD22 se expresa en la superficie de una célula.
51. El anticuerpo de la reivindicación 50, en el que el CD22 es una célula B.
52. El anticuerpo de la reivindicación 1, donde el anticuerpo se une a un epítopo en una región de CD22 de aminoácidos del 22 al 240 del identificador de secuencia n.º: 27.
53. El anticuerpo de la reivindicación 23, donde el anticuerpo se une a un epítopo en una región de CD22 de aminoácidos del 22 al 240 del identificador de secuencia n.º: 27.

54. El anticuerpo de la reivindicación 49, en el que la célula B se asocia a un trastorno proliferativo de células B.

55. El anticuerpo de la reivindicación 54, en el que el trastorno proliferativo de células B es un cáncer.

56. El anticuerpo de la reivindicación 54, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

57. El anticuerpo de la reivindicación 51, en el que la célula B se asocia a un trastorno proliferativo de células B.

58. El anticuerpo de la reivindicación 57, en el que el trastorno proliferativo de células B es un cáncer.

59. El anticuerpo de la reivindicación 57, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto. El anticuerpo de cualquiera de las reivindicaciones entre la 1 y 14, 16 y 23 ó 30 y 33, donde el anticuerpo es un anticuerpo monoclonal.

60. El anticuerpo de la reivindicación 1, en el que el anticuerpo es un anticuerpo monoclonal.

61. El anticuerpo de la reivindicación 60, donde el anticuerpo es un fragmento de anticuerpo seleccionado entre el fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$.

62. El anticuerpo de la reivindicación 60, donde el anticuerpo es humanizado.

63. El anticuerpo de la reivindicación 60, donde el anticuerpo es humano.

64. El anticuerpo de la reivindicación 23, en el que el anticuerpo es un anticuerpo monoclonal.

65. El anticuerpo de la reivindicación 64, donde el anticuerpo es un fragmento de anticuerpo seleccionado entre el fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$.

66. El anticuerpo de la reivindicación 64, donde el anticuerpo es humanizado.

67. El anticuerpo de la reivindicación 64, donde el anticuerpo es humano.

68. El anticuerpo de la reivindicación 1, en el que el anticuerpo se une al mismo epítopo que un anticuerpo seleccionado de ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); y un anticuerpo que comprende una secuencia de la cadena pesada de identificador de secuencia n.°: 88 y una secuencia de la cadena ligera de identificador de secuencia n.°: 87.

69. El anticuerpo de la reivindicación 23, en el que el anticuerpo se une al mismo epítopo que un anticuerpo seleccionado de ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); y un anticuerpo

54. El anticuerpo de la reivindicación 49, en el que la célula B se asocia a un trastorno proliferativo de células B.
55. El anticuerpo de la reivindicación 54, en el que el trastorno proliferativo de células B es un cáncer.
56. El anticuerpo de la reivindicación 54, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.
57. El anticuerpo de la reivindicación 51, en el que la célula B se asocia a un trastorno proliferativo de células B.
58. El anticuerpo de la reivindicación 57, en el que el trastorno proliferativo de células B es un cáncer.
59. El anticuerpo de la reivindicación 57, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

60. El anticuerpo de la reivindicación 1, en el que el anticuerpo es un anticuerpo monoclonal.
61. El anticuerpo de la reivindicación 60, donde el anticuerpo es un fragmento de anticuerpo seleccionado entre el fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$.
62. El anticuerpo de la reivindicación 60, donde el anticuerpo es humanizado.
63. El anticuerpo de la reivindicación 60, donde el anticuerpo es humano.
64. El anticuerpo de la reivindicación 23, en el que el anticuerpo es un anticuerpo monoclonal.
65. El anticuerpo de la reivindicación 64, donde el anticuerpo es un fragmento de anticuerpo seleccionado entre el fragmento Fab, Fab' -SH, Fv, scFv o $(Fab')_2$.
66. El anticuerpo de la reivindicación 64, donde el anticuerpo es humanizado.
67. El anticuerpo de la reivindicación 64, donde el anticuerpo es humano.
68. El anticuerpo de la reivindicación 1, en el que el anticuerpo se une al mismo epítopo que un anticuerpo seleccionado de ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); y un anticuerpo que comprende una secuencia de la cadena pesada de identificador de secuencia n.°: 88 y una secuencia de la cadena ligera de identificador de secuencia n.°: 87.
69. El anticuerpo de la reivindicación 23, en el que el anticuerpo se une al mismo epítopo que un anticuerpo seleccionado de ATCC PTA-7621 (10F4.4.1); ATCC PTA-7620 (5E8.1.8); y un anticuerpo

que comprende una secuencia de la cadena pesada de identificador de secuencia n.°: 88 y una secuencia de la cadena ligera de identificador de secuencia n.°: 87.

70. Un método de detección de la presencia de CD22 en una muestra biológica, dicho método comprende establecer el contacto entre la muestra biológica y un anticuerpo de la reivindicación 1 en condiciones que faciliten la unión del anticuerpo a CD22, y detectar si un complejo está formado por el anticuerpo y CD22.

71. El método de la reivindicación 70, donde la muestra biológica se obtiene de un paciente que se cree que padece un trastorno proliferativo de células B.

72. El método de la reivindicación 71, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

73. Un método de detección de la presencia de CD22 en una muestra biológica, dicho método comprende establecer el contacto entre la muestra biológica y un anticuerpo de la reivindicación 23 en condiciones que faciliten la unión del anticuerpo a CD22, y detectar si un complejo está formado por el anticuerpo y CD22.

74. El método de la reivindicación 73, donde la muestra biológica se obtiene de un paciente que se cree que padece un trastorno proliferativo de células B.

75. El método de la reivindicación 74, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

76. Un inmunoconjugado que comprende un anticuerpo de la reivindicación 1 que se une covalentemente a un agente citotóxico.

77. Un inmunoconjugado que comprende un anticuerpo de la reivindicación 23 que se une covalentemente a un agente citotóxico.

78. El inmunoconjugado de la reivindicación 76, 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.

79. Un inmunoconjugado de la reivindicación 78 que comprende la fórmula Ab-(L-D)p, donde:

(a) Ab es el anticuerpo de la reivindicación 1;

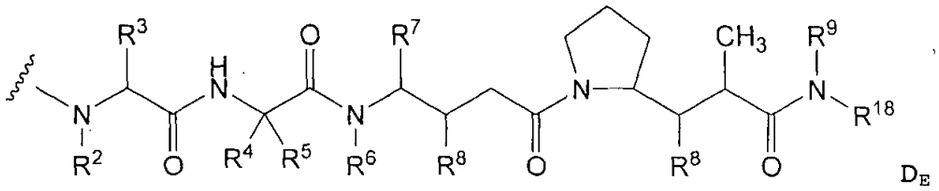
(b) L es un enlazador;

(c) D es una fracción de fármaco.

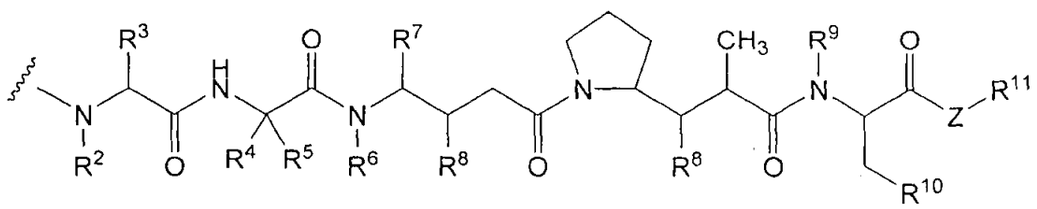
80. El inmunoconjugado de la reivindicación 79, en el que L se selecciona a partir de 6-maleimidocaproilo (MC), maleimidopropanoilo (MP), valina-citrulina (val-cit), alanina-fenilalanina (ala-phe), p-aminobensiloxicarbonil (PAB), N-Succinimidil 4-(2-piridiltio) pentanoato (SPP), N-Succinimidil 4-(N-maleimidometil) ciclohexano-1 carboxilato (SMCC), y N-Succinimidil (4-yodo-acetil) aminobenzoato (SIAB).

81. El inmunoconjugado de la reivindicación 79, en el que D se selecciona a partir de una auristatina y una dolastatina.

82. El inmunoconjugado de la reivindicación 81, 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.

83. El inmunoconjugado de la reivindicación 76, con actividad de destrucción celular in vitro o in vivo.

84. El inmunoconjugado de la reivindicación 79, donde el enlazador se une al anticuerpo a través de un grupo tiol en el anticuerpo.

85. El inmunoconjugado de la reivindicación 79, donde el enlazador es escindible mediante una proteasa.

86. El inmunoconjugado de la reivindicación 80, donde el enlazador comprende un dipéptido val-cit.

87. El inmunoconjugado de la reivindicación 79, donde el enlazador comprende una unidad p-aminobencilo.

88. El inmunoconjugado de la reivindicación 80, donde el enlazador comprende 6-maleimidocaproilo.

89. El inmunoconjugado de la reivindicación 82, donde el fármaco se selecciona entre MMAE y MMAF.

90. El inmunoconjugado de la reivindicación 89, donde el fármaco es MMAE.

91. El inmunoconjugado de la reivindicación 89, donde el fármaco es MMAF.

92. El inmunoconjugado de la reivindicación 77, 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.

93. Un inmunoconjugado de la reivindicación 92 que comprende la fórmula Ab-(L-D)p, donde:

(a) Ab es el anticuerpo de la reivindicación 23;

(b) L es un enlazador;

(c) D es una fracción de fármaco.

94. El inmunoconjugado de la reivindicación 93, en el que L se selecciona a partir de 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).

95. El inmunoconjugado de la reivindicación 93, donde el enlazador L es escindible mediante una proteasa.

96. El inmunoconjugado de la reivindicación 94, donde L comprende un dipéptido val-cit.

97. El inmunoconjugado de la reivindicación 93, donde L comprende una unidad p-aminobencilo.

98. El inmunoconjugado de la reivindicación 97, donde la unidad p-aminobencilo es p-aminobenciloxicarbonilo (PAB).

99. El inmunoconjugado de la reivindicación 94, donde L comprende 6-maleimidocaproilo (MC).

100. El inmunoconjugado de la reivindicación 94, donde el enlazador comprende 6-maleimidocaproilo y p-aminobenziloxicarbonilo.

101. El inmunoconjugado de la reivindicación 79, donde el inmunoconjugado tiene la fórmula

Ab-(L-MMAE)p,

donde L es un enlazador y p oscila entre 2 y 5.

102. El inmunoconjugado de la reivindicación 101, donde L comprende val-cit.

103. El inmunoconjugado de la reivindicación 101, donde L comprende MC.

104. El inmunoconjugado de la reivindicación 101, donde L comprende PAB.

105. El inmunoconjugado de la reivindicación 101, donde L comprende MC-PAB.

106. El inmunoconjugado de la reivindicación 93, donde el inmunoconjugado tiene la fórmula

Ab-(L-MMAE)p,

donde L es un enlazador y p oscila entre 2 y 5.

107. El inmunoconjugado de la reivindicación 106, donde L comprende val-cit.

108. El inmunoconjugado de la reivindicación 106, donde L comprende MC.

109. El inmunoconjugado de la reivindicación 106, donde L comprende PAB.

110. El inmunoconjugado de la reivindicación 106, donde L comprende MC-PAB.

111. El inmunoconjugado de la reivindicación 79, donde el inmunoconjugado tiene la fórmula

Ab-(L-MMAF)p,

donde L es un enlazador y p oscila entre 2 y 5.

112. El inmunoconjugado de la reivindicación 111, donde L comprende val-cit.

113. El inmunoconjugado de la reivindicación 111, donde L comprende MC.

114. El inmunoconjugado de la reivindicación 111, donde L comprende PAB.

115. El inmunoconjugado de la reivindicación 111, donde L comprende MC-PAB.

116. El inmunoconjugado de la reivindicación 93, donde el inmunoconjugado tiene la fórmula

Ab-(L-MMAF)p,

donde L es un enlazador y p oscila entre 2 y 5.

117. El inmunoconjugado de la reivindicación 116, donde L comprende val-cit.

118. El inmunoconjugado de la reivindicación 116, donde L comprende MC.

119. El inmunoconjugado de la reivindicación 116, donde L comprende PAB.

120. El inmunoconjugado de la reivindicación 116, donde L comprende MC-PAB.

121. El inmunoconjugado de la reivindicación 79, donde D es un maitansinoide.

122. El inmunoconjugado de la reivindicación 121, donde D se selecciona a partir de DM1, DM3 y DM4.

123. El inmunoconjugado de la reivindicación 121, con actividad de destrucción celular in vitro o in vivo.

124. El inmunoconjugado de la reivindicación 121, donde el enlazador se une al anticuerpo a través de un grupo tiol en el anticuerpo.

125. El inmunoconjugado de la reivindicación 121, 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).

126. El inmunoconjugado de la reivindicación 122, donde el fármaco es DM1.

127. El inmunoconjugado de la reivindicación 122, donde L comprende un SPP.

128. El inmunoconjugado de la reivindicación 122, donde L comprende SMCC.

129. El inmunoconjugado de la reivindicación 122, donde p oscila entre 2 y 4.

130. El inmunoconjugado de la reivindicación 122, donde p oscila entre 3 y 4.

131. El inmunoconjugado de la reivindicación 93, donde D es un maitansinoide.

132. El inmunoconjugado de la reivindicación 131, donde D se selecciona a partir de DM1, DM3 y DM4.

133. El inmunoconjugado de la reivindicación 131, con actividad de destrucción celular in vitro o in vivo.

134. El inmunoconjugado de la reivindicación 131, donde el enlazador se une al anticuerpo a través de un grupo tiol en el anticuerpo.

135. El inmunoconjugado de la reivindicación 131, 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).

136. El inmunoconjugado de la reivindicación 132, donde el fármaco es DM1.

137. El inmunoconjugado de la reivindicación 136, donde L comprende un SPP.

138. El inmunoconjugado de la reivindicación 136, donde L comprende SMCC.

139. El inmunoconjugado de la reivindicación 136, donde p oscila entre 2 y 4.

140. El inmunoconjugado de la reivindicación 136, donde p oscila entre 3 y 4.

141. Una composición farmacéutica que contiene el inmunoconjugado de la reivindicación 79 y un vehículo farmacéuticamente aceptable.

142. Un método de tratamiento de un trastorno proliferativo de células B que comprenda la administración a un sujeto de una cantidad eficaz de la composición farmacéutica de la reivindicación 141.

143. El método de la reivindicación 142, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

144. Una composición farmacéutica que contiene el inmunoconjugado de la reivindicación 93 y un vehículo farmacéuticamente aceptable.

145. Un método de tratamiento de un trastorno proliferativo de células B que comprenda la administración a un sujeto de una cantidad eficaz de la composición farmacéutica de la reivindicación 144.

146. El método de la reivindicación 145, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

147. Una composición farmacéutica que contiene el inmunoconjugado de las reivindicaciones 76, 77, 78 o92 y un vehículo farmacéuticamente aceptable.

148. Un método de tratamiento de un trastorno proliferativo de células B que comprenda la administración a un sujeto de una cantidad eficaz de la composición farmacéutica de la reivindicación 147.

149. El método de la reivindicación 148, en el que el trastorno proliferativo de células B se selecciona a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

150. Un método para inhibir la proliferación de células B que comprende la exposición de una célula a un inmunoconjugado de la reivindicación 79 en condiciones que faciliten la unión del inmunoconjugado a CD22.

151. El método de la reivindicación 150, en el que la proliferación de células B se asocia a un trastorno seleccionado a partir de los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

152. El método de la reivindicación 150, en el que la célula B es un xenotrasplante.

153. El método de la reivindicación 150, donde la exposición se produce *in vitro*.

154. El método de la reivindicación 150, donde la exposición se produce *in vivo*.

155. El anticuerpo de la reivindicación 1, en el que el anticuerpo es un anticuerpo diseñado con cisteína que comprende uno o más aminoácidos sin cisteína con un valor de reactividad del tiol que oscila entre 0,6 y 1,0, donde el anticuerpo diseñado con cisteína se prepara mediante un proceso que comprende la sustitución de uno o más residuos de aminoácidos de un anticuerpo parental por una cisteína.

156. El anticuerpo diseñado con cisteína de la reivindicación 155, en la que dicho anticuerpo es más reactivo que el anticuerpo parental con un reactivo del tiol.

157. El anticuerpo diseñado con cisteína de la reivindicación 155, en la que el proceso comprende, además, la determinación de la reactividad del tiol del anticuerpo diseñado con cisteína mediante la reacción de dicho anticuerpo con un reactivo del tiol;

donde el anticuerpo diseñado con cisteína es más reactivo que el anticuerpo parental con el reactivo del tiol.

158. El anticuerpo diseñado con cisteína de la reivindicación 155, en el que uno o más residuos de aminoácidos sin cisteína se encuentran en una cadena ligera.

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159. El anticuerpo diseñado con cisteína de la reivindicación 155, en la que el anticuerpo es un inmunoconjugado que comprende el anticuerpo diseñado con cisteína que se une covalentemente a un agente citotóxico.

160. El anticuerpo diseñado con cisteína de la reivindicación 159, 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.

161. El anticuerpo diseñado con cisteína de la reivindicación 155, donde el anticuerpo se une covalentemente a una marca de captura, una marca de detección o un soporte sólido.

162. El anticuerpo diseñado con cisteína de la reivindicación 161, donde el anticuerpo se une covalentemente a una marca de captura de biotina.

163. El anticuerpo diseñado con cisteína de la reivindicación 161, donde el anticuerpo se une covalentemente a una marca de detección de un tinte fluorescente.

164. El anticuerpo diseñado con cisteína de la reivindicación 163, donde el tinte fluorescente se selecciona a partir de un tipo de fluoresceína, un tipo de rodamina, dansil, lisamina, cianina, ficoeritrina, Rojo Texas y un análogo de éstos.

165. El anticuerpo diseñado con cisteína de la reivindicación 161, donde el anticuerpo se une covalentemente a una marca de detección de radionucleidos seleccionada de ^{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 y ^{213}Bi.

166. El anticuerpo diseñado con cisteína de la reivindicación 161, donde el anticuerpo se une covalentemente a una marca de detección mediante un ligando quelante.

167. El anticuerpo diseñado con cisteína de la reivindicación 166, donde el ligando quelante se selecciona a partir de DOTA, DOTP, DOTMA, DTPA y TETA.

168. El anticuerpo de la reivindicación 23, en el que el anticuerpo es un anticuerpo diseñado con cisteína que comprende uno o más aminoácidos sin cisteína con un valor de reactividad del tiol que oscila entre 0,6 y 1,0, donde el anticuerpo diseñado con cisteína se prepara mediante un proceso que comprende la sustitución de uno o más residuos de aminoácidos de un anticuerpo parental por una cisteína.

169. El anticuerpo diseñado con cisteína de la reivindicación 168, en la que dicho anticuerpo es más reactivo que el anticuerpo parental con un reactivo del tiol.

170. El anticuerpo diseñado con cisteína de la reivindicación 168, en la que el proceso comprende, además, la determinación de la reactividad del tiol del anticuerpo diseñado con cisteína mediante la reacción de dicho anticuerpo con un reactivo del tiol;

donde el anticuerpo diseñado con cisteína es más reactivo que el anticuerpo parental con el reactivo del tiol.

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171. El anticuerpo diseñado con cisteína de la reivindicación 168, en el que uno o más residuos de aminoácidos sin cisteína se encuentran en una cadena ligera.

172. El anticuerpo diseñado con cisteína de la reivindicación 168, en la que el anticuerpo es un inmunoconjugado que comprende el anticuerpo diseñado con cisteína que se une covalentemente a un agente citotóxico.

173. El anticuerpo diseñado con cisteína de la reivindicación 172, 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.

174. El anticuerpo diseñado con cisteína de la reivindicación 168, donde el anticuerpo se une covalentemente a una marca de captura, una marca de detección o un soporte sólido.

175. El anticuerpo diseñado con cisteína de la reivindicación 174, donde el anticuerpo se une covalentemente a una marca de captura de biotina.

176. El anticuerpo diseñado con cisteína de la reivindicación 174, donde el anticuerpo se une covalentemente a una marca de detección de un tinte fluorescente.

177. El anticuerpo diseñado con cisteína de la reivindicación 176, donde el tinte fluorescente se selecciona a partir de un tipo de fluoresceína, un tipo de rodamina, dansil, lisamina, cianina, ficoeritrina, Rojo Texas y un análogo de éstos.

178. El anticuerpo diseñado con cisteína de la reivindicación 174, donde el anticuerpo se une covalentemente a una marca de detección de radionucleidos seleccionada de ^{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 y ^{213}Bi.

179. El anticuerpo diseñado con cisteína de la reivindicación 178, donde el anticuerpo se une covalentemente a una marca de detección mediante un ligando quelante.

180. El anticuerpo diseñado con cisteína de la reivindicación 179, donde el ligando quelante se selecciona a partir de DOTA, DOTP, DOTMA, DTPA y TETA.

181. El inmunoconjugado de la reivindicación 93, donde el enlazador se une al anticuerpo a través de un grupo tiol en el anticuerpo.

182. El anticuerpo de la reivindicación 1 comprende un péptido de unión a la albúmina.

183. El anticuerpo de la reivindicación 182, donde el péptido de unión a la albúmina se selecciona a partir de los identificadores de secuencia n.º: 42 al 46.

184. El anticuerpo de la reivindicación 23 comprende un péptido de unión a la albúmina.

185. El anticuerpo de la reivindicación 184, donde el péptido de unión a la albúmina se selecciona a partir de los identificadores de secuencia n.º: 42 al 46.

186. El anticuerpo de la reivindicación 1, donde el anticuerpo comprende, además, una cisteína en una o más posiciones seleccionadas de 15, 43, 110, 144, 168 y 205 de la cadena ligera según la convención

numérica de Kabat y 41, 88, 115, 118, 120, 171, 172, 282, 375 y 400 de la cadena pesada según la convención numérica de EU.

187. El anticuerpo de la reivindicación 186, donde una cisteína se encuentra en la posición 205 de la cadena ligera.

188. El anticuerpo de la reivindicación 186, donde una cisteína se encuentra en la posición 118 de la cadena pesada.

189. El anticuerpo de la reivindicación 186, donde una cisteína se encuentra en la posición 400 de la cadena pesada.

190. El anticuerpo de la reivindicación 186, donde el anticuerpo se selecciona a partir de un anticuerpo monoclonal, un anticuerpo biespecífico, un anticuerpo quimérico, un anticuerpo humano y un anticuerpo humanizado.

191. El anticuerpo de la reivindicación 186 que es un fragmento de anticuerpo.

192. El anticuerpo de la reivindicación 191, donde el fragmento de anticuerpo es un fragmento Fab.

193. El anticuerpo de la reivindicación 186 que se selecciona a partir de un anticuerpo quimérico, un anticuerpo humano o un anticuerpo humanizado.

194. El anticuerpo de la reivindicación 186 que se produce en una bacteria.

195. El anticuerpo de la reivindicación 186 que se produce en células CHO.

196. Un método para determinar la presencia de una proteína CD22 en una muestra que se cree que contiene dicha proteína; este método comprende exponer esta muestra a un anticuerpo de la reivindicación 186 y determinar la unión de este anticuerpo a dicha proteína CD22 en esta muestra, donde la unión del anticuerpo a dicha proteína indica la presencia de dicha proteína en la muestra.

197. El método de la reivindicación 196, donde la muestra comprende una célula que se cree que expresa dicha proteína CD22.

198. El método de la reivindicación 196, donde dicha célula es una célula B.

199. El método de la reivindicación 196, donde el anticuerpo se une covalentemente a una marca seleccionada a partir de un tinte fluorescente, un radioisótopo, una biotina o un ligando de formación de complejos de metal.

200. Una formulación farmacéutica que comprende el anticuerpo anti-CD22 de la reivindicación 186 y un diluyente, vehículo o excipiente farmacéuticamente aceptable.

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201. El anticuerpo de la reivindicación 186, donde el anticuerpo se une covalentemente a una fracción del fármaco auristatina o maitansinoide, a través de la cual se forma el conjugado de anticuerpo con fármacos.

202. El conjugado de anticuerpo con fármacos de la reivindicación 201 comprende un anticuerpo (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 sin cisteína y de una porción de enlazador (L) a D, el compuesto que posee Fórmula I.

$$Ab–(L–D)_p \qquad I$$

donde p es 1, 2, 3 ó 4.

203. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 201, donde p es 2.

204. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 201, 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,

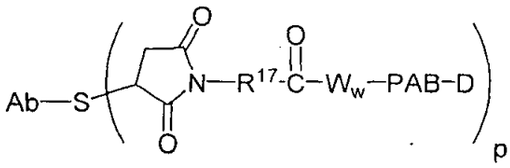
cada W es independientemente una unidad de aminoácidos,

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.

205. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 204 con 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)–$(CH_2)_r$–, $–(CH_2)_rC(O)NR^b(CH_2)_r–$, $–(CH_2CH_2O)_r–$, $–(CH_2CH_2O)_r–CH_2–$, $–(CH_2)_rC(O)NR^b(CH_2CH_2O)_r–$, $–(CH_2)_rC(O)NR^b(CH_2CH_2O)_r–CH_2–$, $–(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r–$,

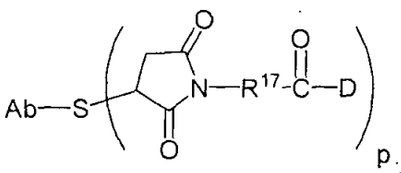
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$-(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r-CH_2-$ y $-(CH_2CH_2O)_rC(O)NR^b(CH_2)_t-$; donde R^b es H, C_1-C_6 alquilo, fenilo o bencilo y r es independientemente un número entero que oscila entre 1 y 10.

206. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 204, donde W_w es valina-citrulina.

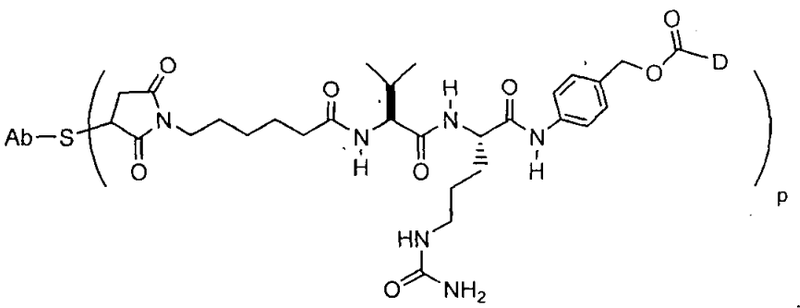
207. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 204, donde ^{17}R es $(CH_2)_5$ o $(CH_2)_2$.

208. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 204 con la fórmula:



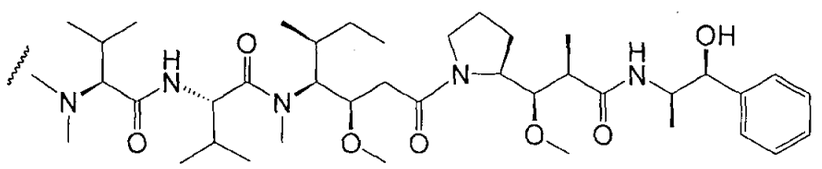
209. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 208, donde ^{17}R es $(CH_2)_5$ o $(CH_2)_2$.

210. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 204 con la fórmula:



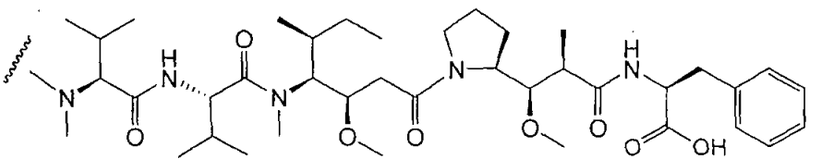
211. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 202, donde L es SMCC, SPP o BMPEO.

212. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 202, donde D es MMAE con la estructura:



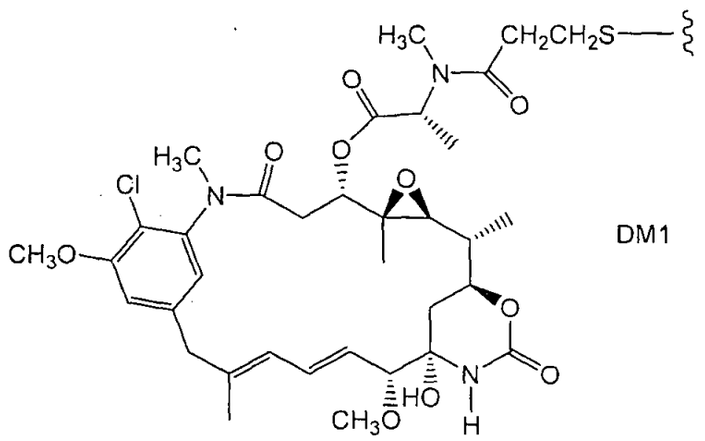
donde la línea ondulada indica el sitio de unión al enlazador L.

213. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 202, donde D es MMAF con la estructura:



donde la línea ondulada indica el sitio de unión al enlazador L.

214. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 202, donde D es DM1 con la estructura:

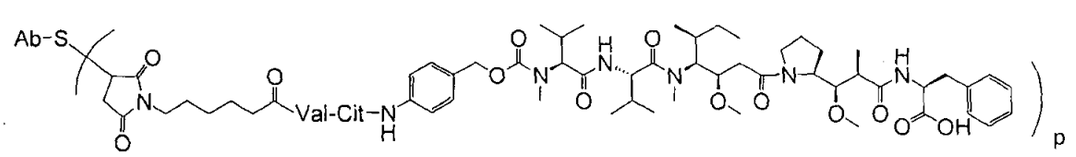


donde la línea ondulada indica el sitio de unión al enlazador L.

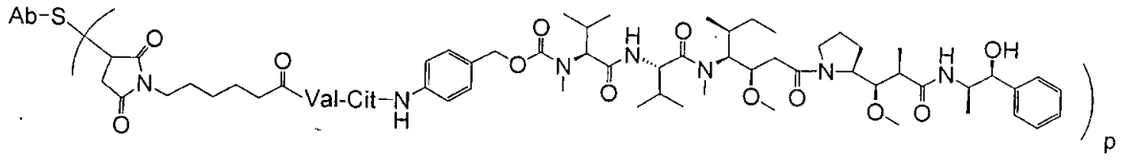
215. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 201, donde el anticuerpo anti-CD22 parental se selecciona a partir de un anticuerpo monoclonal, un anticuerpo biespecífico, un anticuerpo quimérico, un anticuerpo humano, un anticuerpo humanizado y un fragmento de anticuerpo.

216. El compuesto de conjugado de anticuerpo con fármacos de la reivindicación 201, donde el fragmento de anticuerpo es un fragmento Fab.

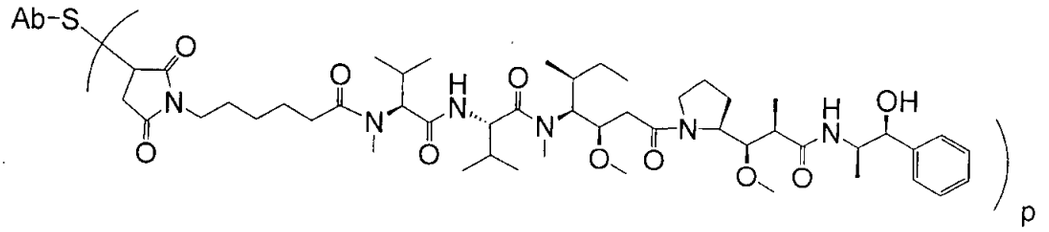
217. El compuesto de conjugado de anticuerpo con fármacos seleccionado de las estructuras:



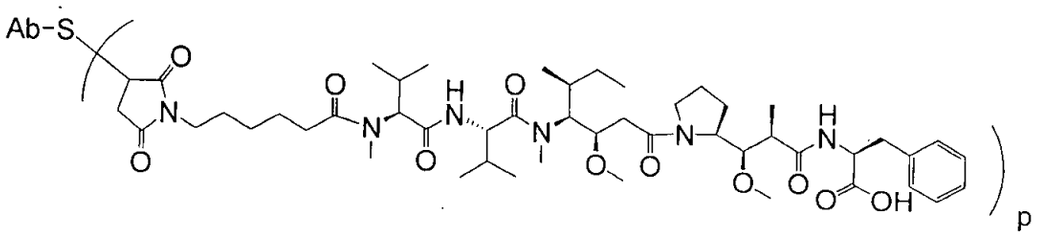
Ab-MC-vc-PAB-MMAF



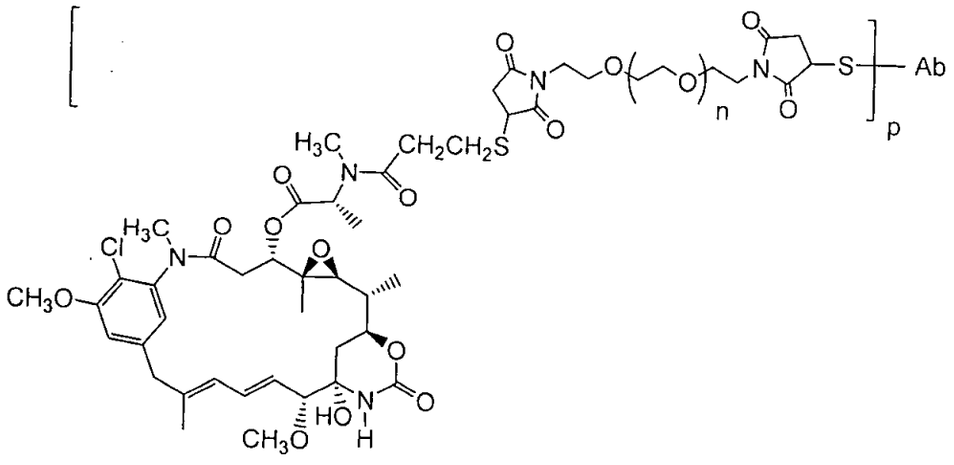
Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF



Ab-BMPEO-DM1

un envase y un prospecto o etiqueta que indica que el compuesto puede utilizarse para tratar el cáncer caracterizado por la sobreexpresión de un polipéptido CD22.

227. El elemento de fabricación de la reivindicación 226, el que el cáncer se selecciona a partir del grupo que comprende los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

228. El método para que un compuesto de conjugado de anticuerpo con fármacos comprenda un anticuerpo anti-CD22 (Ab) de la reivindicación 186, y una fracción del fármaco auristatina o maitansinoide (D) en la que el anticuerpo se une por medio de uno o más aminoácidos diseñados con cisteína y de una porción de enlazador (L) a D, el compuesto que posee Fórmula I.

$$Ab-(L-D)_p \qquad \mathbf{I}$$

donde p es 1, 2, 3 ó 4; el método comprende los pasos para que:

(a) un grupo de anticuerpos diseñados con cisteína reaccione con un reactivo enlazador para formar un anticuerpo enlazador intermedio Ab-L, y

(b) Ab-L reaccione con una fracción de fármaco D activada, donde se forma el conjugado de anticuerpo con fármacos,

o comprende los pasos para que:

(c) un grupo nucleofílico de una fracción de fármaco reaccione con un reactivo enlazador para formar un enlazador a fármacos intermedio D-L, y

(d) D-L reaccione con un grupo de anticuerpos diseñados con cisteína, donde se forma el conjugado de anticuerpo con fármacos.

229. El método de la reivindicación 228 comprende, además, el paso para expresar el anticuerpo en células de ovario de hámster chino (CHO).

230. El método de la reivindicación 229 también comprende el paso para tratar el anticuerpo expresado con un agente reductor.

231. El método de la reivindicación 230, donde el agente reductor se selecciona a partir de TCEP y DTT.

232. El método de la reivindicación 230 también comprende el paso para tratar el anticuerpo expresado con un agente oxidante, después de tratarlo con un agente reductor.

233. El método de la reivindicación 232, donde el agente oxidante se selecciona a partir de un sulfato de cobre, un ácido dehidroascórbico y del aire.

234. El anticuerpo de la reivindicación 186, donde el anticuerpo comprende una secuencia de la cadena pesada seleccionada a partir de cualquiera de los identificadores de secuencia n.°: 88, 92 ó 93.

235. El anticuerpo de la reivindicación 186, donde el anticuerpo comprende una secuencia de la cadena ligera seleccionada a partir de cualquiera de los identificadores de secuencia n.°: 87 ó 91

236. El anticuerpo de la reivindicación 186, donde el anticuerpo comprende una secuencia de la cadena ligera de identificador de secuencia n.°: 87 y una secuencia de la cadena pesada de identificador de secuencia n.°: 92.

237. El anticuerpo de la reivindicación 186, donde el anticuerpo comprende una secuencia de la cadena ligera de identificador de secuencia n.°: 87 y una secuencia de la cadena pesada de identificador de secuencia n.°: 93.

238. El anticuerpo de la reivindicación 186, donde el anticuerpo comprende una secuencia de la cadena ligera de identificador de secuencia n.°: 91 y una secuencia de la cadena pesada de identificador de secuencia n.°: 88.

donde Val es valina, Cit es citrulina, p es 1, 2, 3 ó 4 y Ab es anticuerpo anti-CD22 de la reivindicación 186.

218. El conjugado de anticuerpo con fármacos de la reivindicación 201, donde la auristatina es MMAE o MMAF.

219. El conjugado de anticuerpo con fármacos de la reivindicación 202, donde L es MC-val-cit-PAB o MC.

220. Un ensayo para detectar las células B que comprende:

(a) exponer las células a un compuesto de conjugado de anticuerpo con fármacos de la reivindicación 199, y

(b) determinar el grado de unión del compuesto de conjugado de anticuerpo con fármacos a la células.

221. Un método para inhibir la proliferación celular que comprende tratar las células B cancerosas de mamíferos en un medio de cultivo celular con un compuesto de conjugado de anticuerpo con fármacos de la reivindicación 201, donde se inhibe la proliferación de las células B cancerosas.

222. Una formulación farmacéutica que comprende un conjugado de anticuerpo con fármacos de la reivindicacion 201 y un diluyente, vehículo o excipiente farmacéuticamente aceptable.

223. Un método para tratar el cáncer que comprende la administración a un paciente de una formulación farmacéutica de la reivindicación 222.

224. El método de la reivindicación 223, en el que el cáncer se selecciona a partir del grupo que comprende los linfoma, linfoma no Hogkins (LNH), LNH agresivo, LNH agresivo recurrente, LNH indolente recurrente, LNH refractario, LNH indolente refractario, leucemia linfocítica crónica (LLC), linfoma linfocítico de células pequeñas, leucemia, leucemia de células pilosas (LCP), leucemia linfocítica aguda (LLA) y linfoma de células del manto.

225. El método de la reivindicación 223, donde se administra al paciente un agente citotóxico en combinación con un compuesto de conjugado de anticuerpo con fármacos.

226. Un artículo fabricado que comprende

una formulación farmacéutica de la reivindicación 220,

RESUMEN DEL DESCUBRIMIENTO

Se proporcionan anticuerpos anti-CD22 y sus inmunoconjugados. Se proporcionan métodos de uso de los anticuerpos anti-CD22 y de sus inmunoconjugados.

5

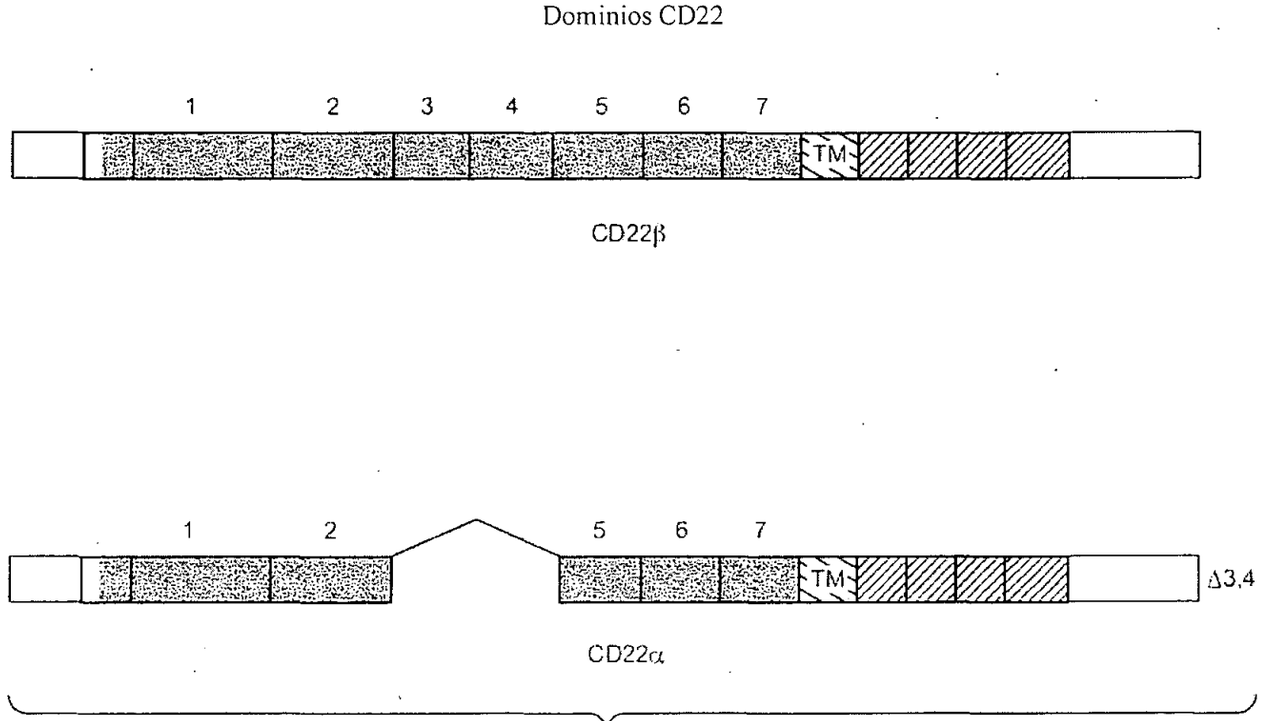


FIG. 1A

633

```
   1 mhllgpwlll lvleylafsd sskwvfehpe tlyawegacv wipctyrald gdlesfilfh
  61 npeynkntsk fdgtrlyest kdgkvpseqk rvqflgdknk nctlsihpvh lndsgqlglr
 121 mesktekwme rihlnvserp fpphiqlppe iqesqevtlt cllnfscygy piqlqwlleg
 181 vpmrqaavts tsltiksvft rselkfspqw shhgkivtcq lqdadgkfls ndtvqlnvkh
 241 tpkleikvtp sdaivregds vtmtcevsss npeyttvswl kdgtslkkqn tftlnlrevt
 301 kdqsgkyccq vsndvgpgrs eevflqvqya pepstvqilh spavegsqve flcmslanpl
 361 ptnytwyhng kemqgrteek vhipkilpwh agtyscvaen iigtggrqpg aeldvqyppk
 421 kvttviqnpm piregdtvtl scnynssnps vtryewkphg aweepslgvl kignvgwdnt
 481 tiacarcnsw cswaspvaln vqyaprdvrv rkikplseih sgnsvslqcd fsasnpkevq
 541 ffwekngrll gkesqlnfds ispedagsys cwvnnsiggt askawtlevl yaprrlrvsm
 601 spgdqvmegk satltcesda nppvshytwf dwnnqsiphh sqklrlepvk vqhsgaywcq
 661 gtnsvgkgrs plstltvyys petigrrvav glgsclaill laicglklql rwkrtqsqqg
 721 lqenssgqsf fvrnkkvrra plsegphslg cynpmmedgi syttlrfpem niprtgdaes
 781 semqrpprtc ddtvtysalh krqvgdyenv ipdfpedegi hyseliqfgv gerpqaqenv
 841 dyvilkh (identificador de sencuencia n.º: 27)
```

FIG. 1B

```
   1 mhllgpwlll lvleylafsd sskwvfehpe tlyawegacv wipctyrald gdlesfilfh
  61 npeynkntsk fdgtrlyest kdgkvpseqk rvqflgdknk nctlsihpvh lndsgqlglr
 121 mesktekwme rihlnvserp fpphiqlppe iqesqevtlt cllnfscygy piqlqwlleg
 181 vpmrqaavts tsltiksvft rselkfspqw shhgkivtcq lqdadgkfls ndtvqlnvkh
 241 ppkkvttviq npmpiregdt vtlscnynss npsvtryewk phgaweepsl gvlkignvgw
 301 dnttiacaac nswcswaspv alnvqyaprd vrvrkikpls eihsgnsvsl qcdfsasnpk
 361 evqffwekng rllgkesqln fdsispedag syscwvnnsi ggtaskawtl evlyaprrlr
 421 vsmspgdqvm egksatltce sdanppvshy twfdwnnqsl pyhsqklrle pvkvqhsgay
 481 wcqgtnsvgk grsplstltv yyspetigrr vavglgscla illlaicglk lqrrwkrtqs
 541 qqglqenssg qsffvrnkkv rraplsegph slgcynpmme dgisyttlrf pemniprtgd
 601 aessemqrpp pdcddtvtys alhkrqvgtm rtsfqifqkm rgfitqs (identificador de sencuencia n.º: 29)
```

FIG. 1C

```
MHLLGFWLLLLEYLAFSDS SKWNIEHPGTIYAWEGACIWVPCTYRVLDGA
LETFILFHNPEYNQNMSKFEGTRLYESTKDGKVPSGQKRVQFLGNKINNNC
TLSIHPVHVNDSGQLGLRMVSKTEKWMERIHLNVSERPFPPRIQLPPKLQE
SQEVTLTCLLNFSCYGYQIQLQWLLEGAPMRQAAVTLTSLSTKSVFTRSEL
KFSPQWSHHGKIVTCELHDVDGKVLSEDTVQLNVKHTPKLTIEVTPNETIV
RKGDSVTMTCKVNSSNPEYTTVSWLKDDIPLKEQNTLMLTLHEVTKSQTGT
YCCRVSNDVGPATSEKVFLQVQYAPEPSRVQISQSPAVEGSEVNFLCISPA
NFLPTNYTWYHNGKEVQGRTEKQFQIQKILPWHAGTYSCVAENILGIGERG
PGTELDVQYPPKKVTIMVIENPTPIREGDTVTLSCNYSSSNPIVNHYEWRPR
GAWEEPSLGVLKIQNIGWNNTAVACAACNNNCSWASPVTLNVLYAPRGVRV
RKIKPLSEIHSGNLVSLQCDFSSSHPKEVQFFWEKNGSLLGKESQLNFDSI
SPEDAGSYSCWVNNSIGQTASKAWTLEVLYAPRRLRVSMSQGNQVMEGKTA
ILTCESDANPPVYSYAWFDWNNQSLPYSGRMLRLEPVKVQHSGAYWCQGTN
RVGKGHSPLITLTVYYSPETIGRRVAVGLGSCLAILILAMCGFKVQRRNKR
TQSQQGLQENSSGQSFFVRNKKVRRTPLSEGPHSLGCYNPMMEDGISYATL
RFPETNTPRTGDAETSELQRLPPDCDDTVTYSVLQKRQVGDYENVIPDFPE
DEGIHYSELIQFGVGERPQAQENVDYVIVKH (identificador de sencuencia n.º: 31)
```

FIG. 1D

Dominio variable de la cadena pesada
Alineación de secuencias de las variantes anti-CD22 10F4

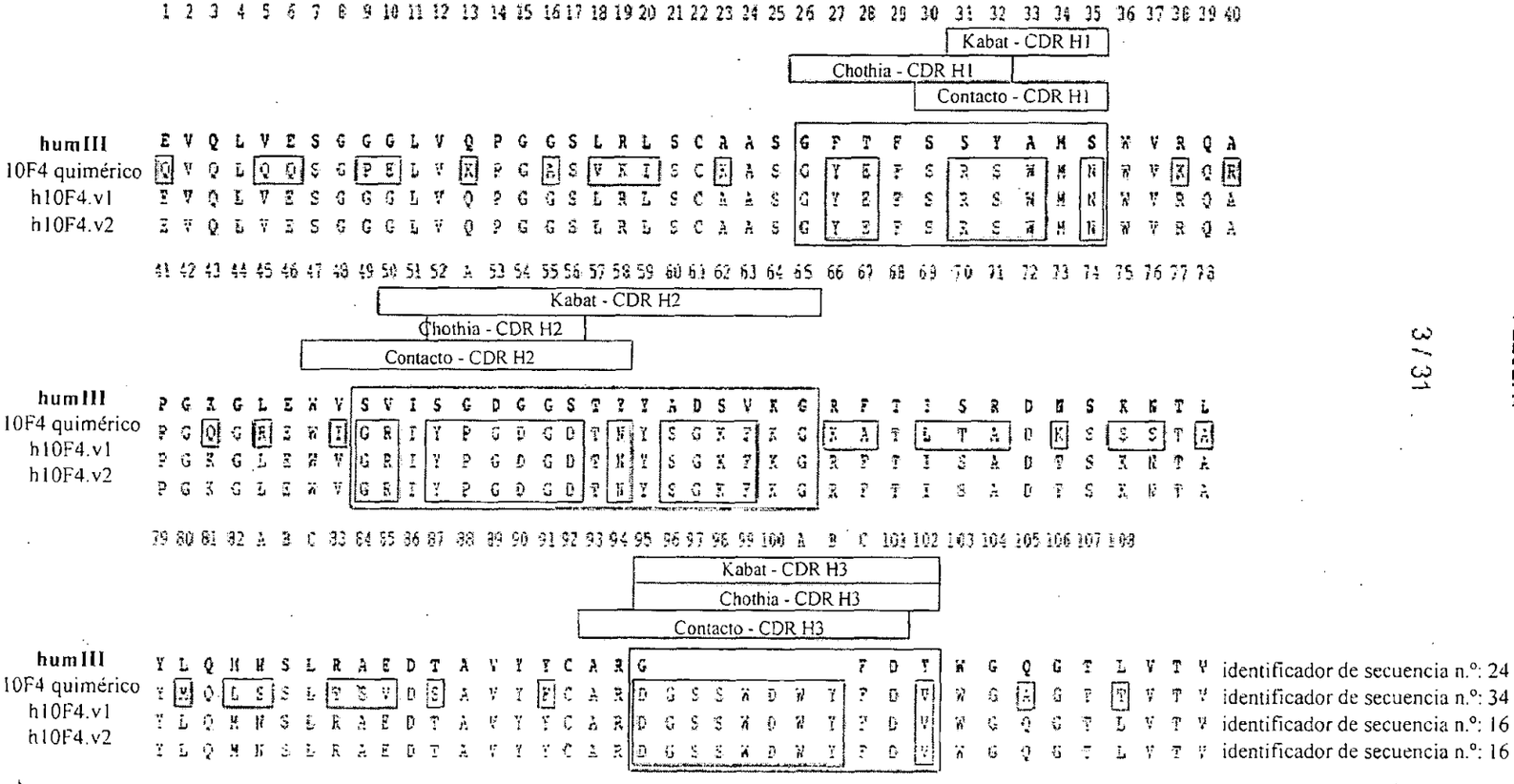


FIG. 2A

Dominio variable de la cadena ligera
Alineación de secuencias de las variantes anti-CD22 10F4

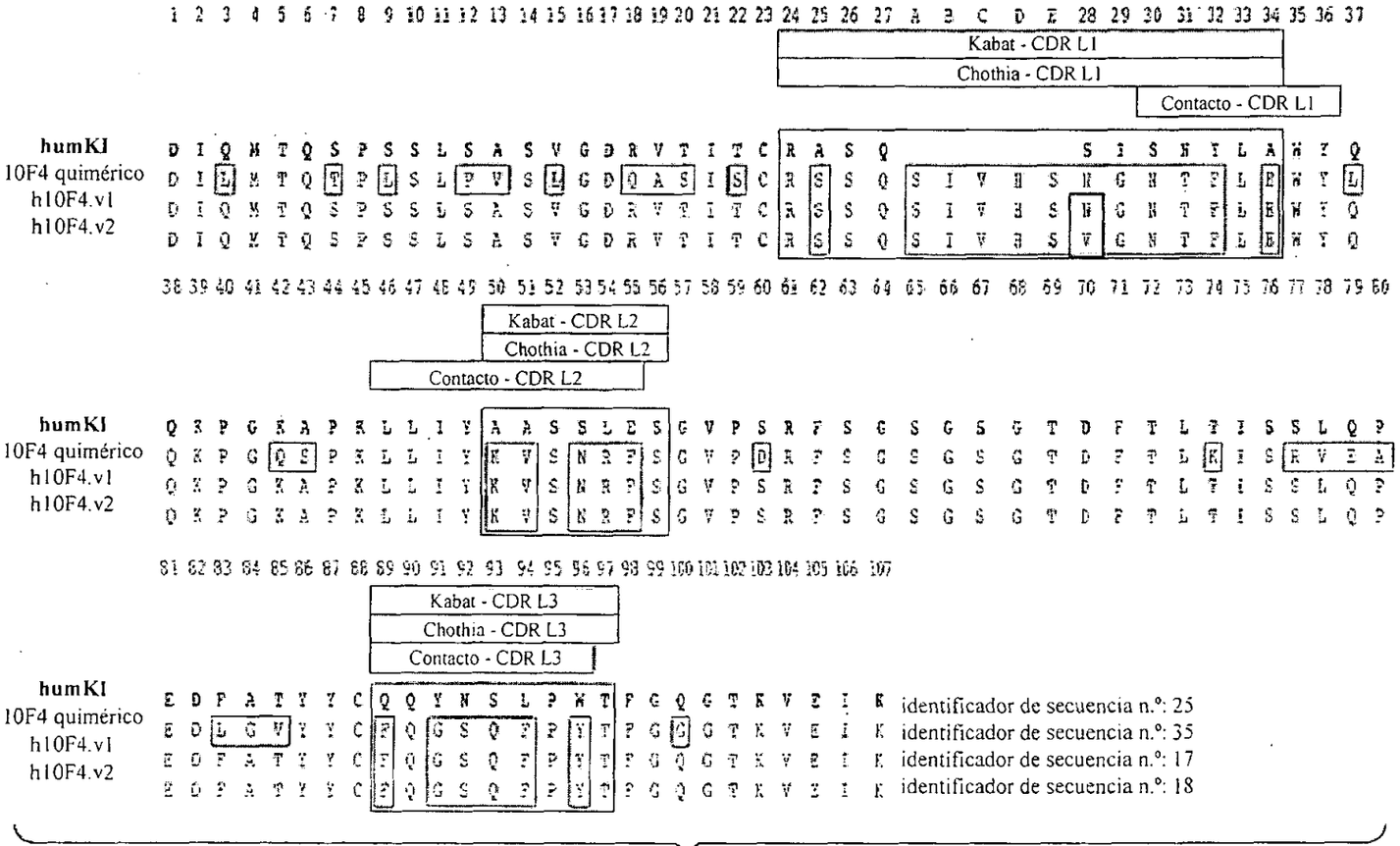


FIG. 2B

636

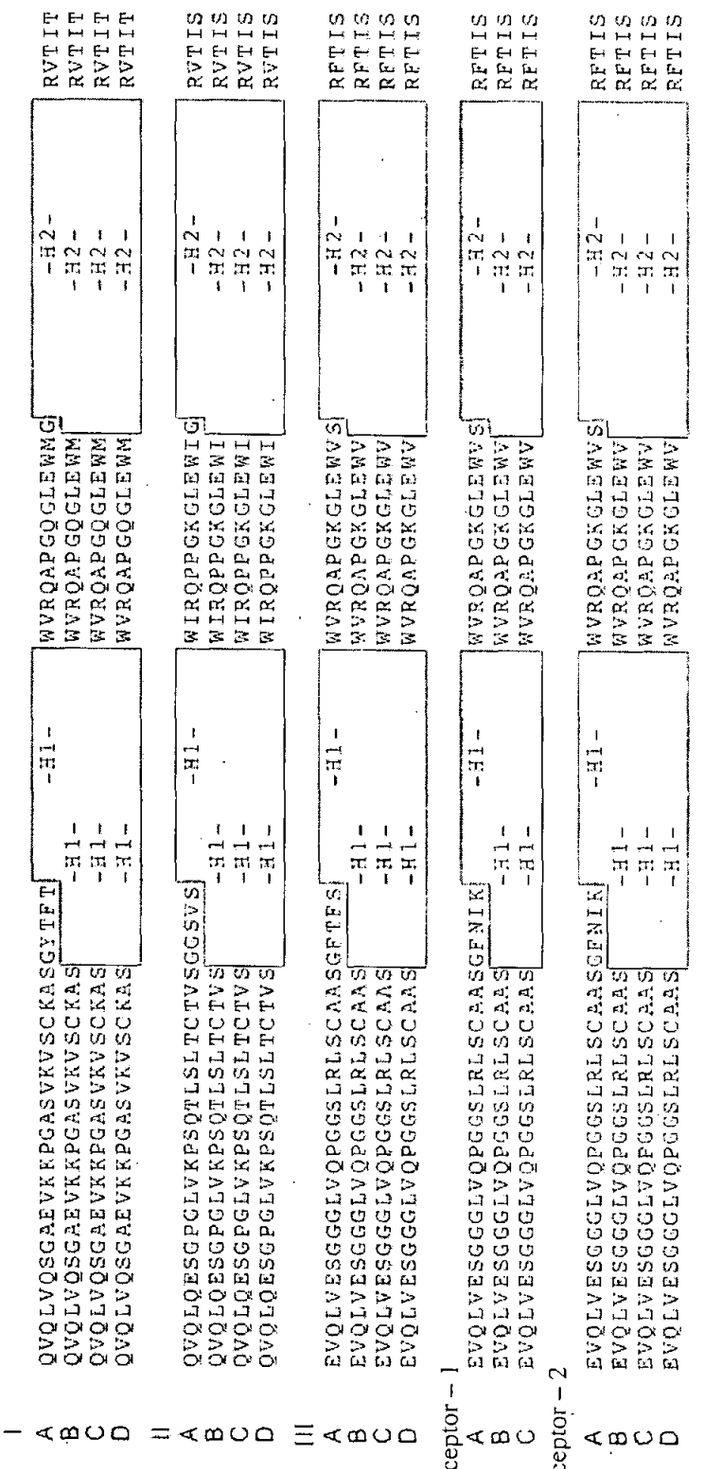

I
A QVQLVQSGAEVKKPGASVKVSCKASGYTFT -H1- WVRQAPGQGLEWMG -H2- RVTIT
B QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
C QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
D QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
II
A QVQLQESGPGLVKPSQTLSLTCTVSGGSVS -H1- WIRQPPGKGLEWIG -H2- RVTIS
B QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
C QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
D QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
III
A EVQLVESGGGLVQPGGSLRLSCAASGFTFS -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Aceptor – 1
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Aceptor – 2
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK -H1- WVRQAPGKGLEWVS -H2- RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS

FIG. 3A

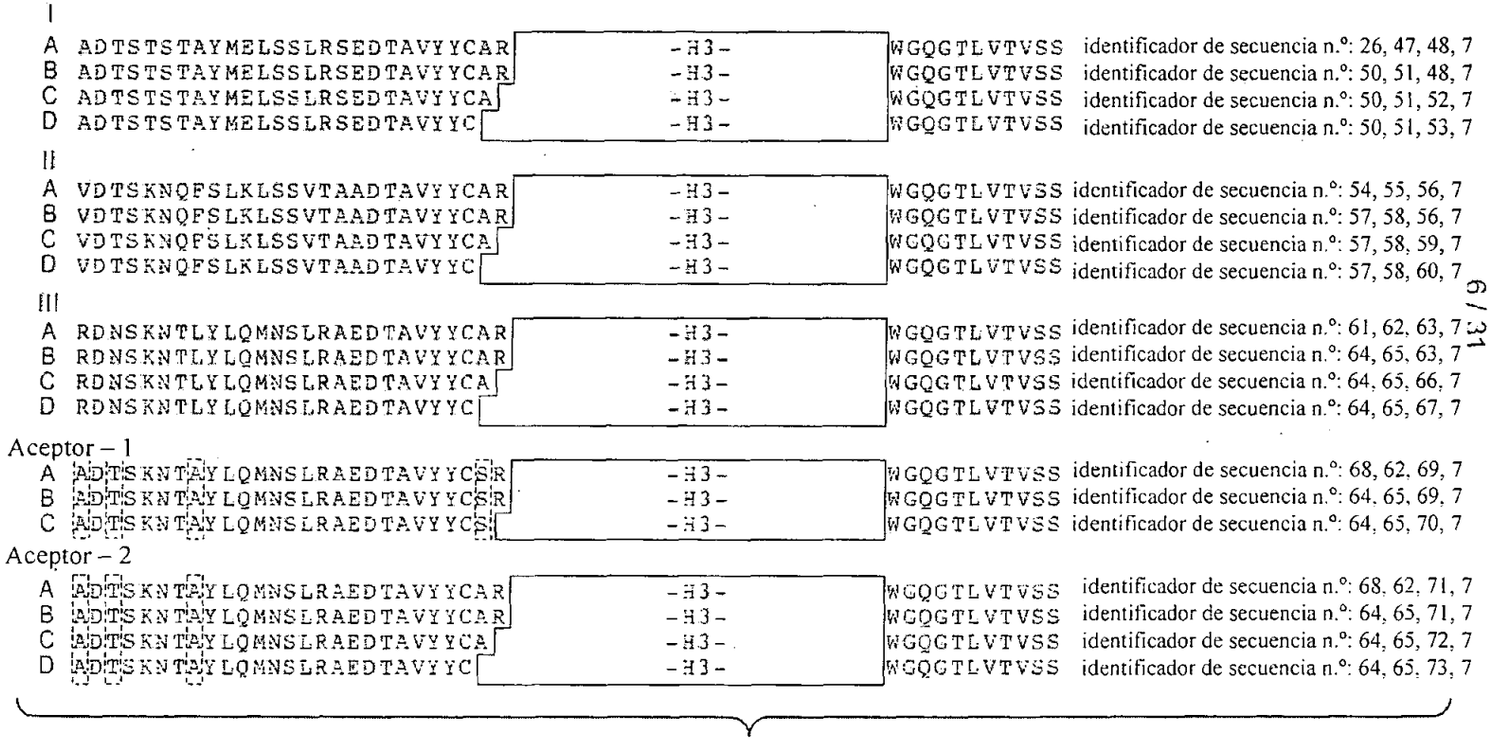

I
A ADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 26, 47, 48, 7
B ADTSTSTAYMELSSLRSEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 50, 51, 48, 7
C ADTSTSTAYMELSSLRSEDTAVYYCA -H3- WGQGTLVTVSS identificador de secuencia n.º: 50, 51, 52, 7
D ADTSTSTAYMELSSLRSEDTAVYYC -H3- WGQGTLVTVSS identificador de secuencia n.º: 50, 51, 53, 7
II
A VDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 54, 55, 56, 7
B VDTSKNQFSLKLSSVTAADTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 57, 58, 56, 7
C VDTSKNQFSLKLSSVTAADTAVYYCA -H3- WGQGTLVTVSS identificador de secuencia n.º: 57, 58, 59, 7
D VDTSKNQFSLKLSSVTAADTAVYYC -H3- WGQGTLVTVSS identificador de secuencia n.º: 57, 58, 60, 7
III
A RDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 61, 62, 63, 7
B RDNSKNTLYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 63, 7
C RDNSKNTLYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 66, 7
D RDNSKNTLYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 67, 7
Aceptor – 1
A ADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS identificador de secuencia n.º: 68, 62, 69, 7
B ADTSKNTAYLQMNSLRAEDTAVYYCSR -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 69, 7
C ADTSKNTAYLQMNSLRAEDTAVYYCS -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 70, 7
Aceptor – 2
A ADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 68, 62, 71, 7
B ADTSKNTAYLQMNSLRAEDTAVYYCAR -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 71, 7
C ADTSKNTAYLQMNSLRAEDTAVYYCA -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 72, 7
D ADTSKNTAYLQMNSLRAEDTAVYYC -H3- WGQGTLVTVSS identificador de secuencia n.º: 64, 65, 73, 7

FIG. 3B

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kv1 DIQMTQSPSSLSASVGDRVTITC[-L1-]WYQQKPGKAPKLLIY[-L2-]GVPSRFSGSGSGTDFTLTISSLQP

kv1 DIQMTQSPSSLSASVGDRVTITC[-L1-]WYQQKPGKAPKLLI[-L2-]GVPSRFSGSGSGTDFTLTISSLQP

kv2 DIVMTQSPLSLPVTPGEPASISC[-L1-]WYLQKPGQSPQLLIY[-L2-]GVPDRFSGSGSGTDFTLKISRVEA

kv3 EIVLTQSPGTLSLSPGERATLSC[-L1-]WYQQKPGQAPRLLIY[-L2-]GIPDRFSGSGSGTDFTLTISRLEP

kv4 DIVMTQSPDSLAVSLGERATINC[-L1-]WYQQKPGQPPKLLIY[-L2-]GVPDRFSGSGSGTDFTLTISSLQA

FIG. 4A

kv1 EDFATYYC[-L3-]FGQGTKVEIK identificador de secuencia n.º: 74, 75, 76, 77

kv1 EDFATYYC[-L3-]FGQGTKVEIK identificador de secuencia n.º: 74, 78, 76, 77

kv2 EDVGVYYC[-L3-]FGQGTKVEIK identificador de secuencia n.º: 49, 79, 80, 77

kv3 EDFAVYYC[-L3-]FGQGTKVEIK identificador de secuencia n.º: 81, 82, 83, 77

kv4 EDVAVYYC[-L3-]FGQGTKVEIK identificador de secuencia n.º: 84, 85, 86, 77

FIG. 4B

```
          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  identificador de secuencia n.º: 38
humIgG2     EALHNHYTQKSLSLSPGK  identificador de secuencia n.º: 39
humIgG3     EALHNRFTQKSLSLSPGK  identificador de secuencia n.º: 40
humIgG4     EALHNHYTQKSLSLSLGK  identificador de secuencia n.º: 41
                  **         *
```

Una secuencia de consenso de la región constante de la cadena ligera Kappa:

```
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC  Identificador de secuencia n.º: 37
```

FIG. 5A

Cadena ligera de anti-CD22 h10F4v2

DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKPGKAPKLLI
YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSQFPYTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC (Identificador de secuencia n.°: 87)

Cadena pesada de anti-CD22 h10F4v2

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (Identificador de secuencia n.°: 88)

FIG. 5B

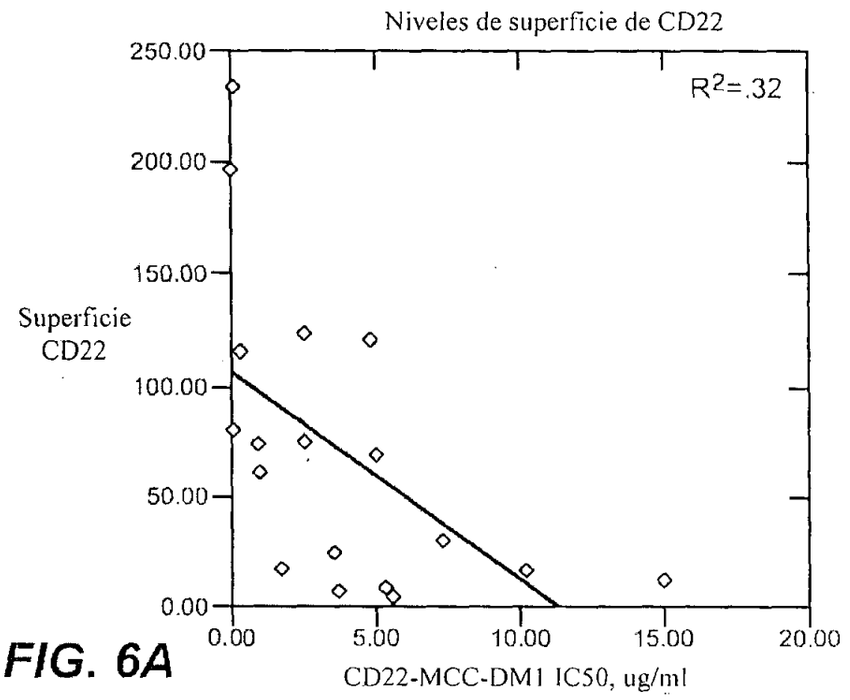

Niveles de superficie de CD22
R^2=.32
Superficie CD22
250.00
200.00
150.00
100.00
50.00
0.00
0.00
5.00
10.00
15.00
20.00
CD22-MCC-DM1 IC50, ug/ml

FIG. 6A

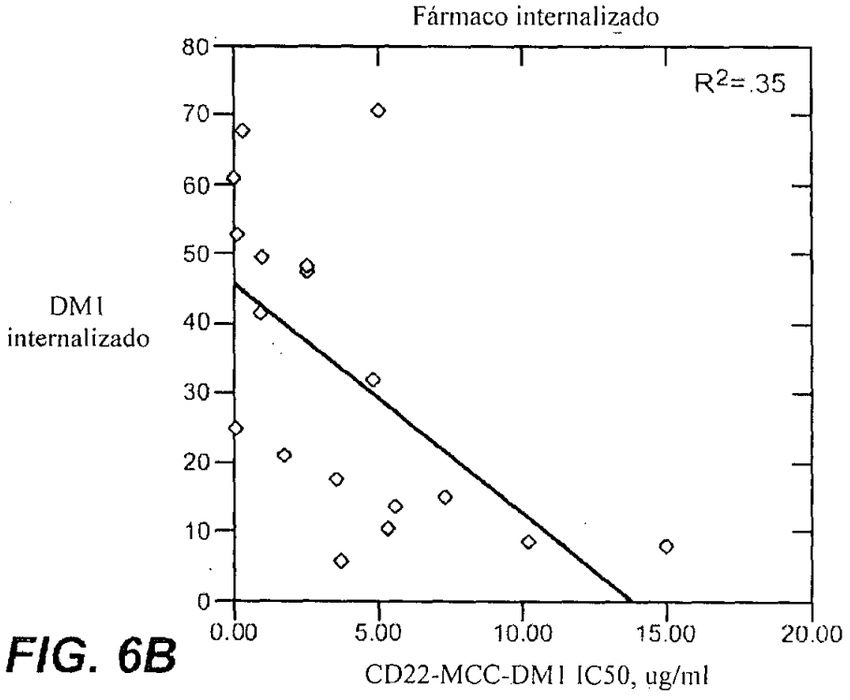

Fármaco internalizado
R^2=.35
DM1 internalizado
80
70
60
50
40
30
20
10
0
0.00
5.00
10.00
15.00
20.00
CD22-MCC-DM1 IC50, ug/ml

FIG. 6B

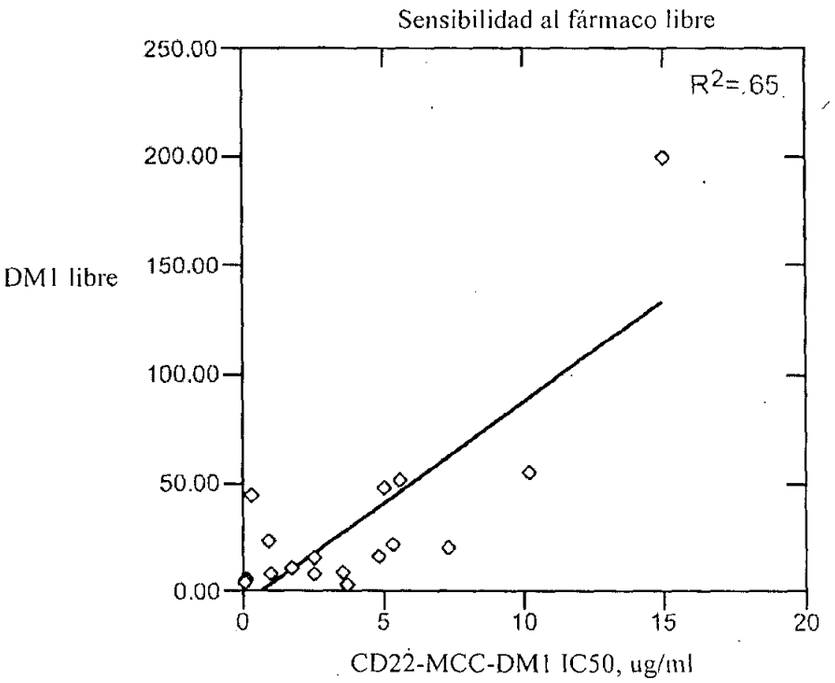

Sensibilidad al fármaco libre
250.00
200.00
150.00
100.00
50.00
0.00
DM1 libre
R^2=.65
0
5
10
15
20
CD22-MCC-DM1 IC50, ug/ml

FIG. 6C

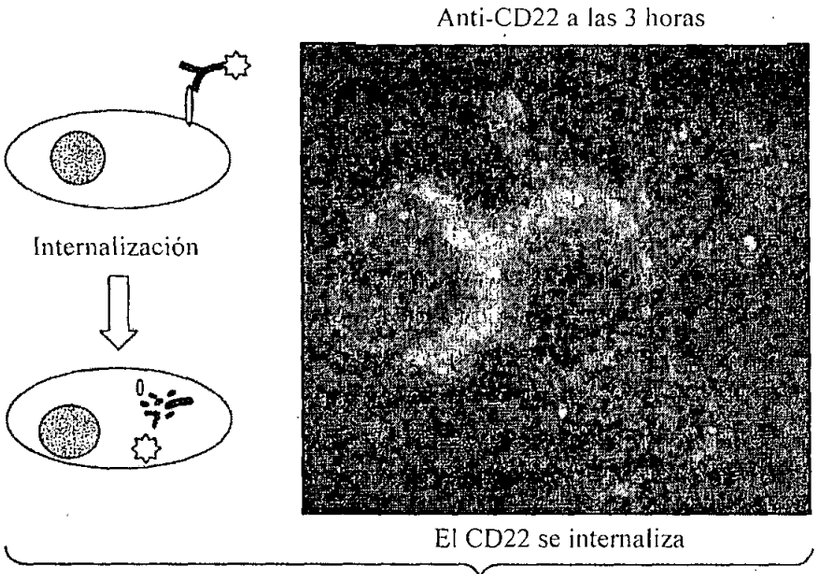

Anti-CD22 a las 3 horas
Internalización
El CD22 se internaliza

FIG. 6D

Anti-CD22(10F4v1)-smcc-DM1 en modelos xenotrasplantados BJAB-luc

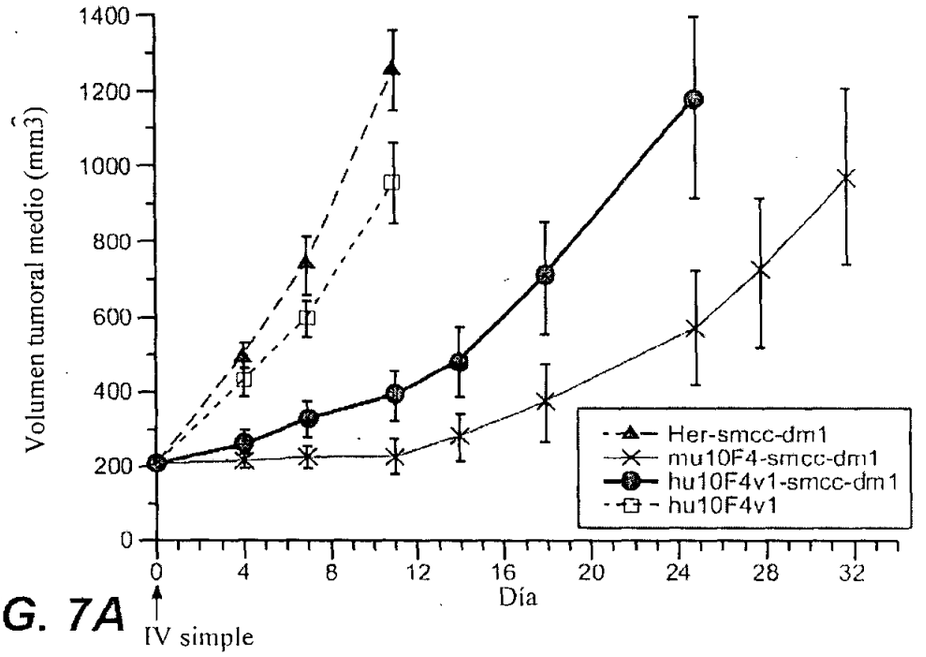


FIG. 7A

Anti-CD22(10F4v1)-smcc-DM1 y anticuerpos desnudos en el modelo xenotrasplantado BJAB-luc

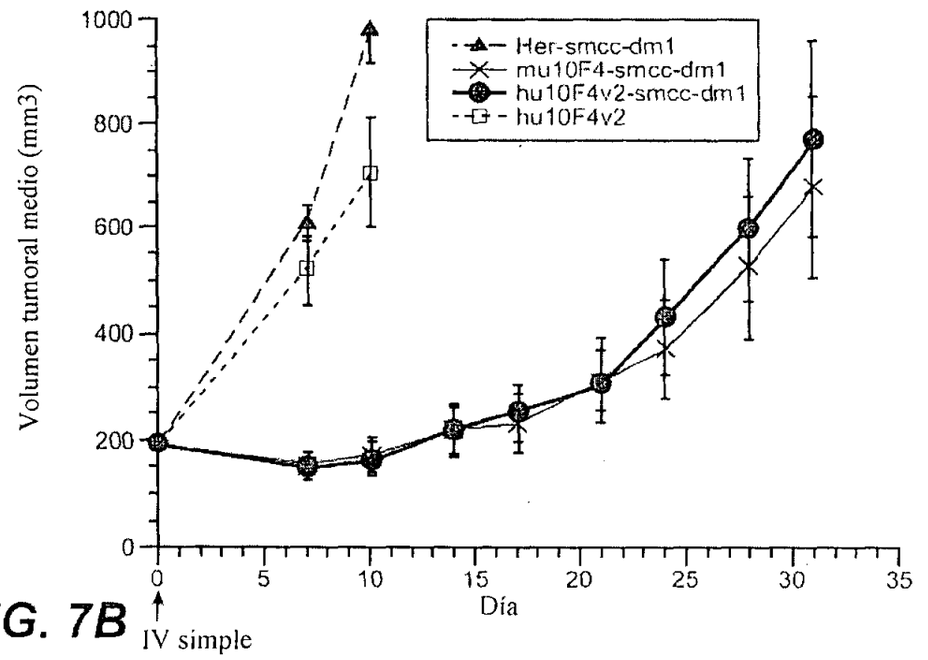


FIG. 7B

Anti-CD22-spp-DM1 and –smcc-DM1 en modelos xenotrasplantados BJAB-luc

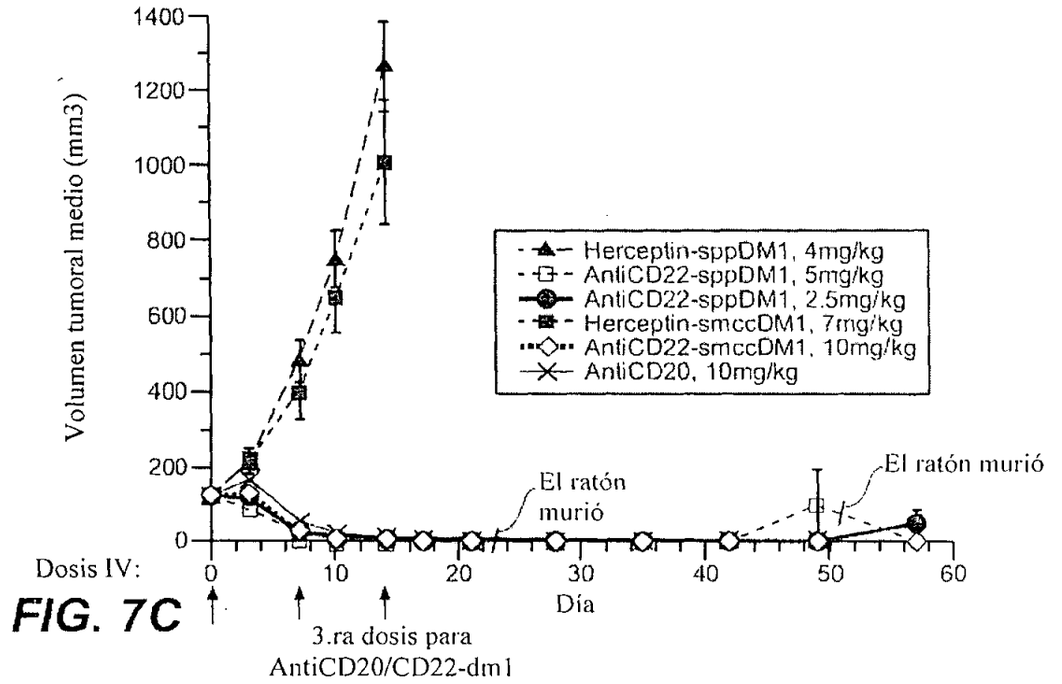


FIG. 7C

Anti-CD22-smcc-DM1 en modelos xenotrasplantados Ramos

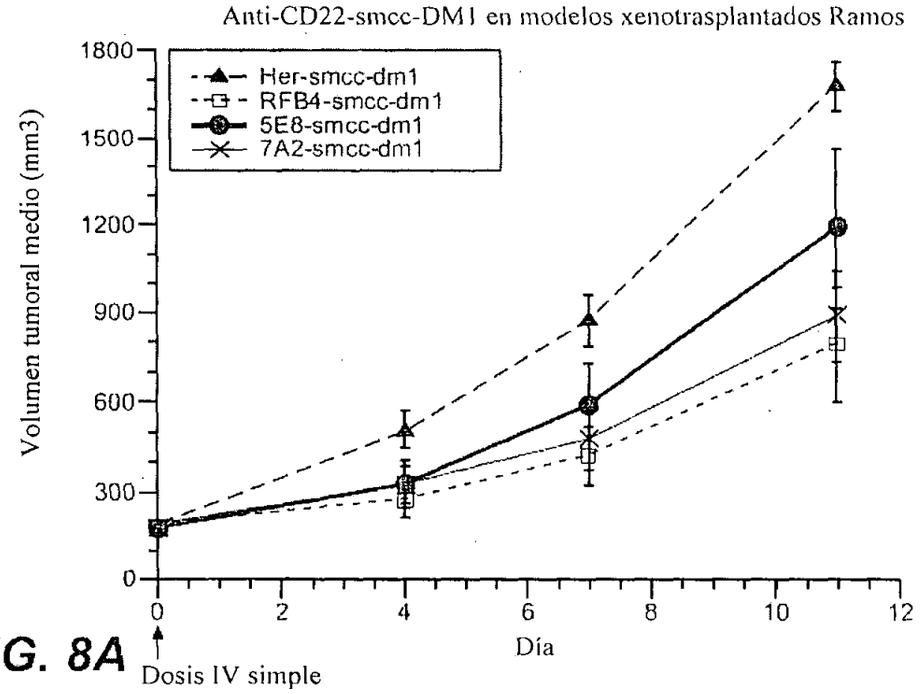


FIG. 8A

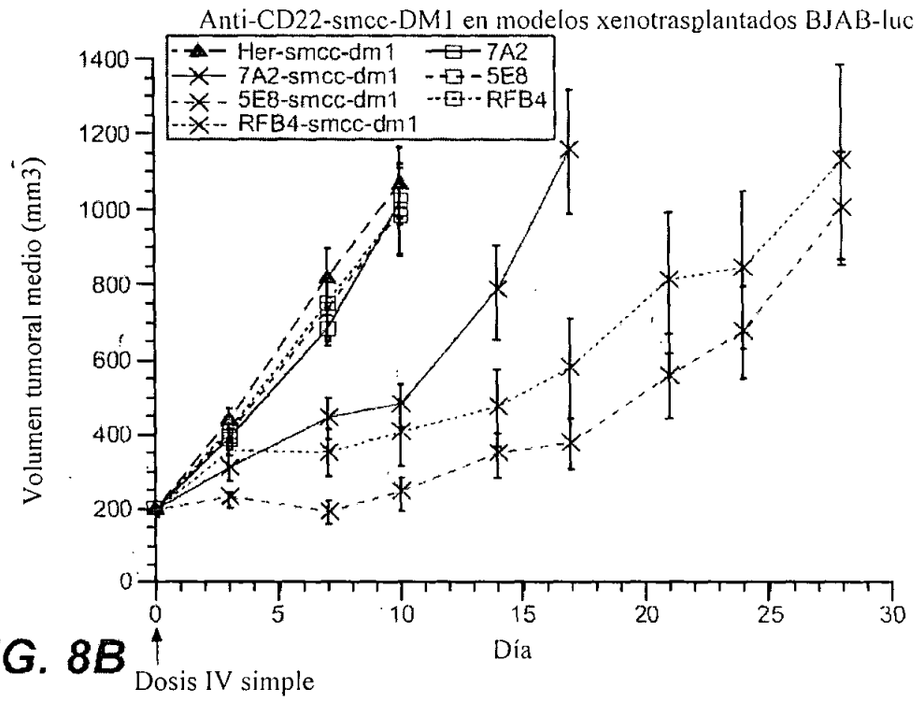

Anti-CD22-smcc-DM1 en modelos xenotrasplantados BJAB-luc
Her-smcc-dm1
7A2
7A2-smcc-dm1
5E8
5E8-smcc-dm1
RFB4
RFB4-smcc-dm1
Volumen tumoral medio (mm3)
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Día
Dosis IV simple

FIG. 8B

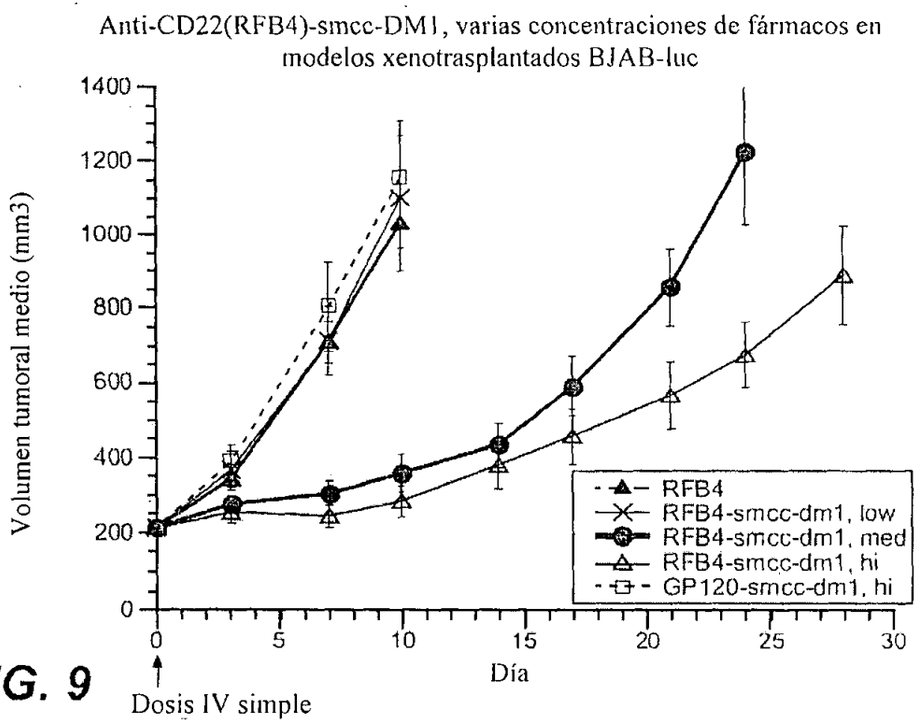

Anti-CD22(RFB4)-smcc-DM1, varias concentraciones de fármacos en modelos xenotrasplantados BJAB-luc
Volumen tumoral medio (mm3)
1400
1200
1000
800
600
400
200
0
RFB4
RFB4-smcc-dm1, low
RFB4-smcc-dm1, med
RFB4-smcc-dm1, hi
GP120-smcc-dm1, hi
0
5
10
15
20
25
30
Día
Dosis IV simple

FIG. 9

Conjugado de Anti-CD22 con MMAF en modelos xenotrasplantados Ramos

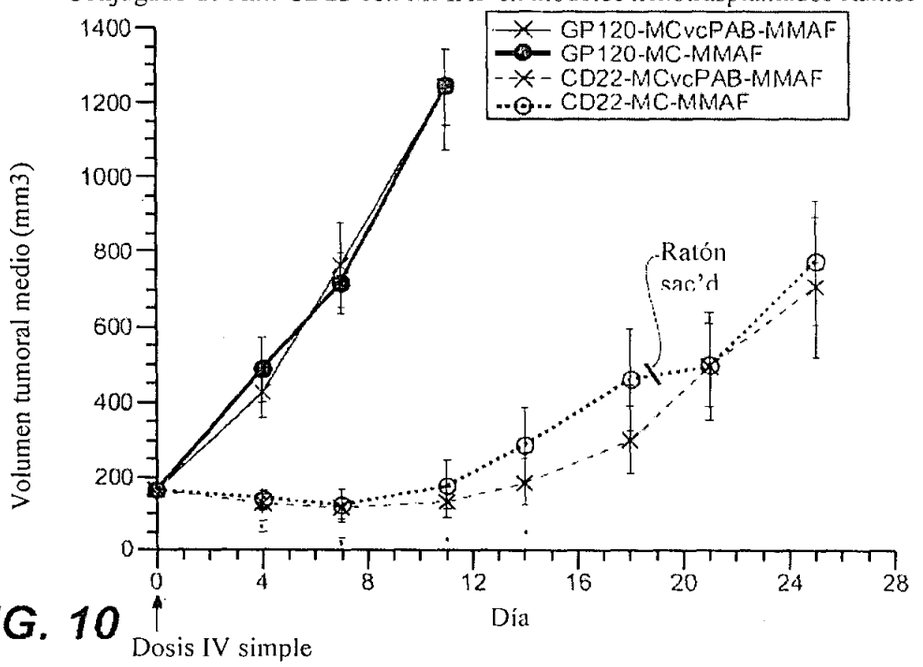


FIG. 10

Conjugado de Anti-CD22 con MMAE y DM1 en modelos xenotrasplantados Ramos

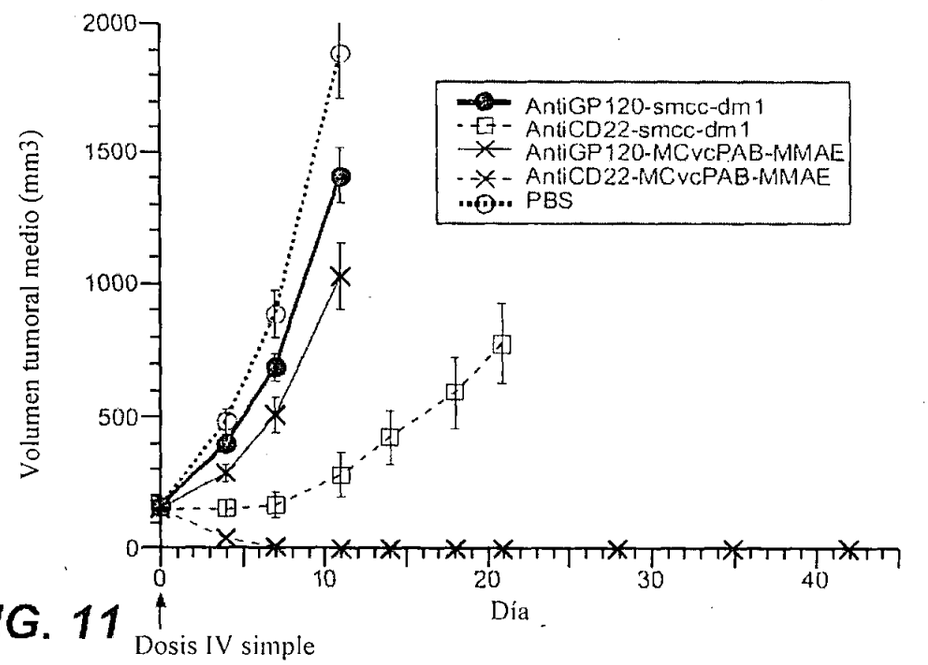


FIG. 11

ADC de Anti-CD22 hu10F4 con DM1 y MMAF en modelos xenotrasplantados BJAB-luc

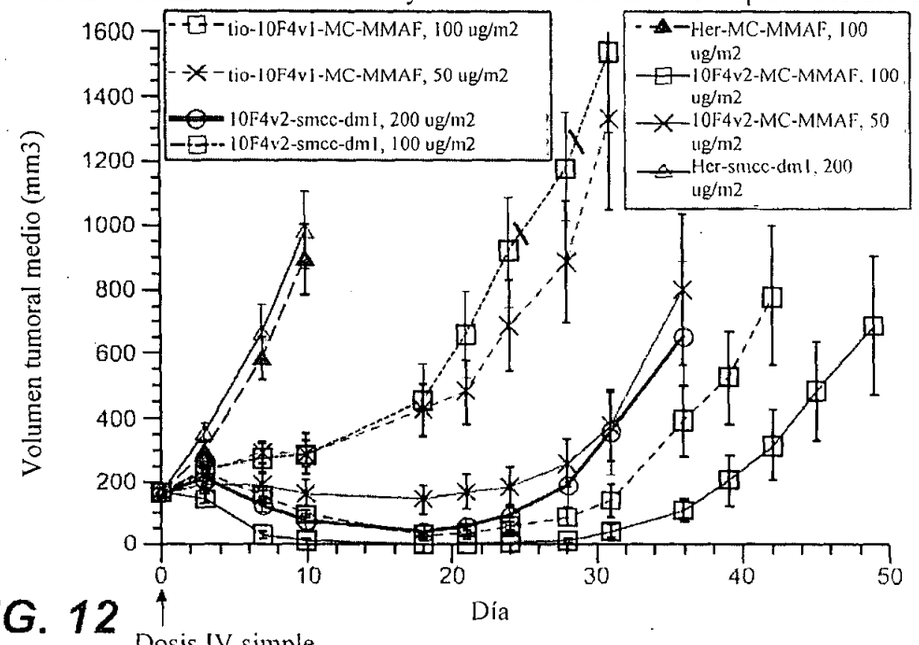


FIG. 12

ADC de Anti-CD22 hu10F4v3 con DM1 y MMAF en modelos xenotrasplantados SuDHL4-luc

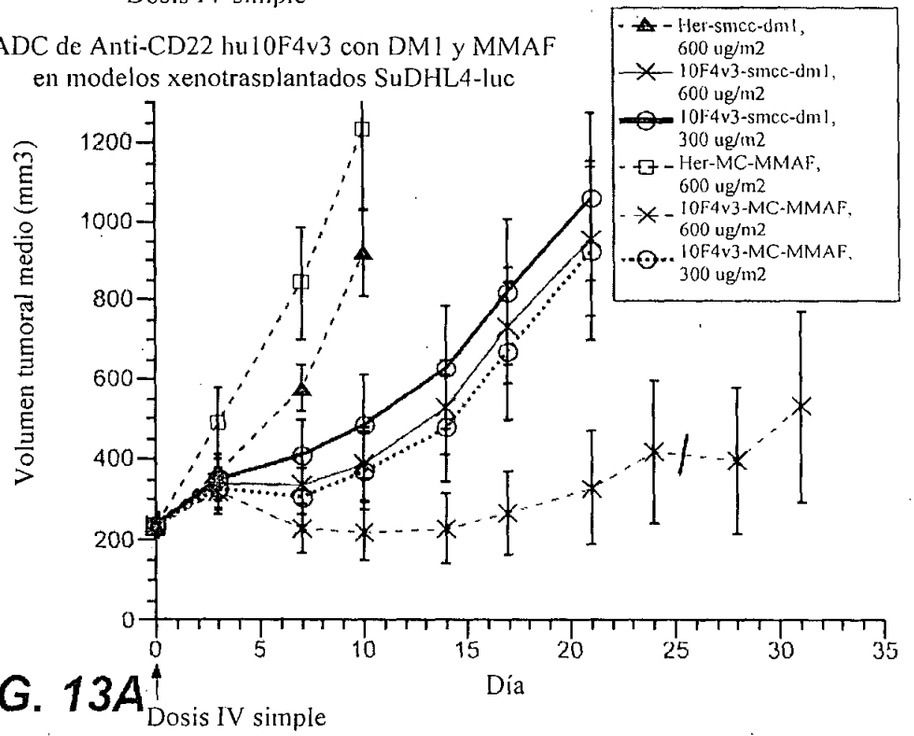


FIG. 13A

ADC de Anti-CD22 hu10F4v3 con DM1y MMAF en modelos xenotrasplantados DoHH2

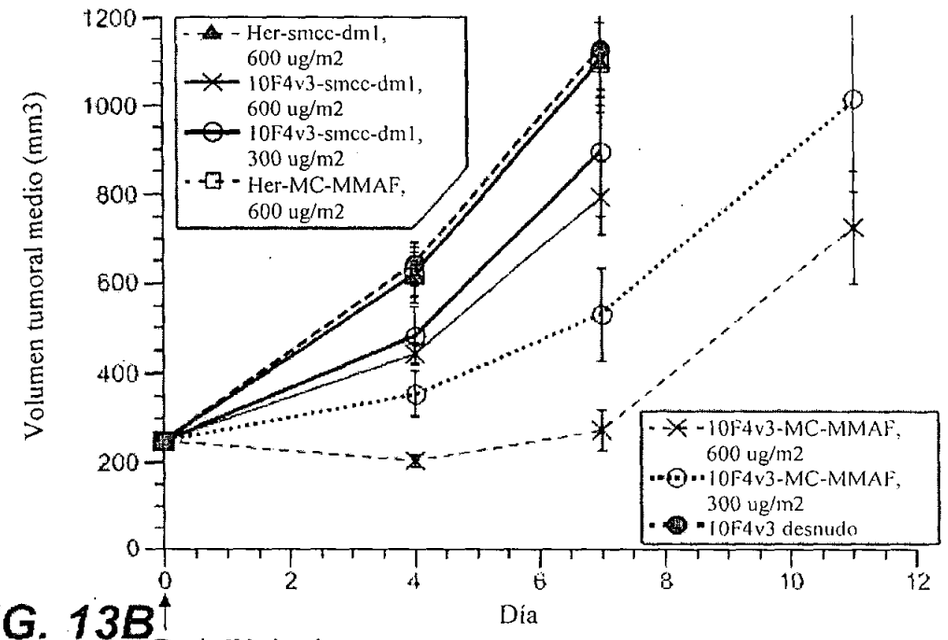


FIG. 13B

Dosis IV simple

ADC de Anti-CD22 hu10F4v3 con DM1 y MMAF en modelos xenotrasplantados Granta-519

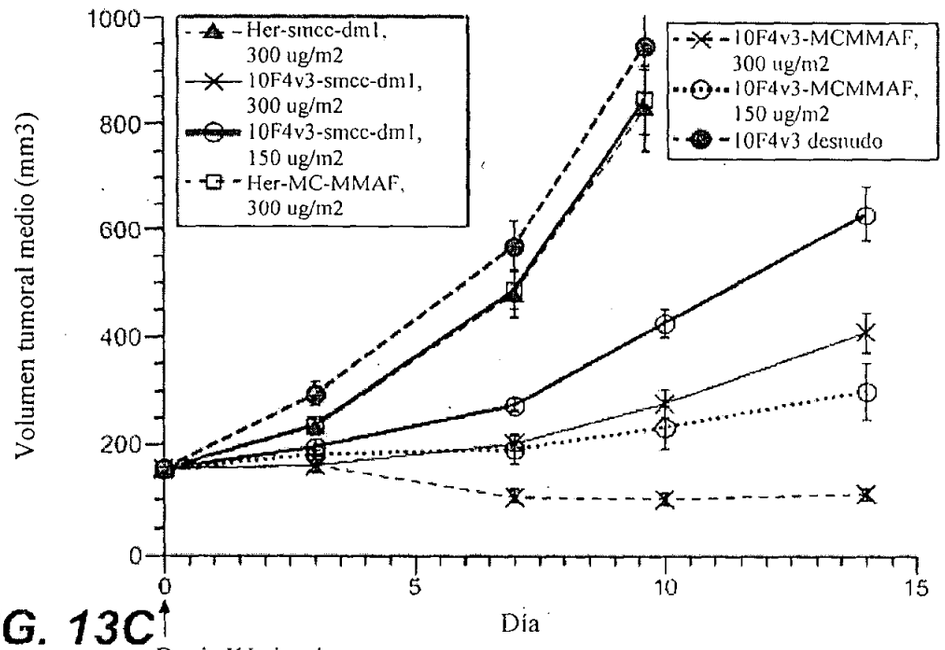


FIG. 13C

Dosis IV simple

Eliminaciones de los dominios CD22 para el mapeo de epítopos

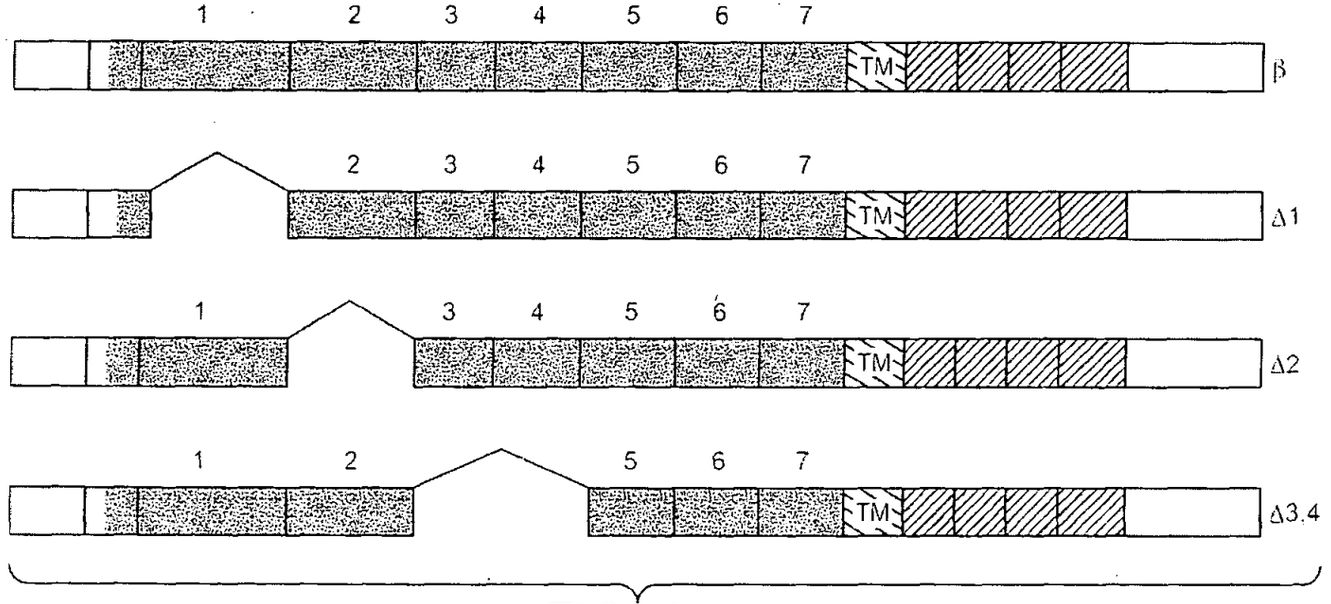


FIG. 14

ES

FIG. 15

LC-ADC
HC-ADC
Fc-ADC
Fracción de fármaco

FIG. 16

Reducción
Oxidación
Conjugación
ThioMab
Reducido
Reoxidado
ADC de tio

Cadena ligera de anti-CD22 V205C h10F4v3 diseñado con cisteína

DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKPGKAPKLLI
YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCFQGSQFPYTFGQG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPCTK
SFNRGEC (Identificador de secuencia n.º: 91)

FIG. 17A

Cadena pesada de anti-CD22 A118C h10F4v3 diseñado con cisteína

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSCSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (Identificador de secuencia n.º: 92)

FIG. 17B

Región Fc de anti-CD22 S400C h10F4v3 diseñado con cisteína

EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPGKGLEWVGRIYP
GDGDTNYSGKFKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARDGSSWDWY
FDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK (Identificador de secuencia n.º: 93)

FIG. 17C

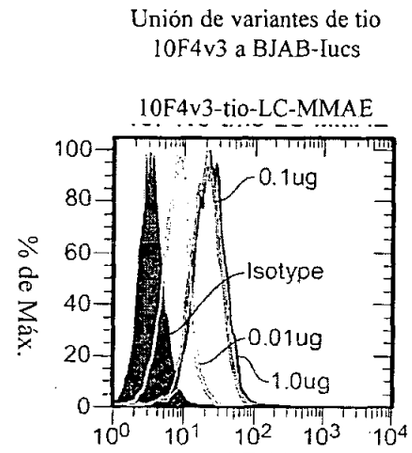


FIG. 18A

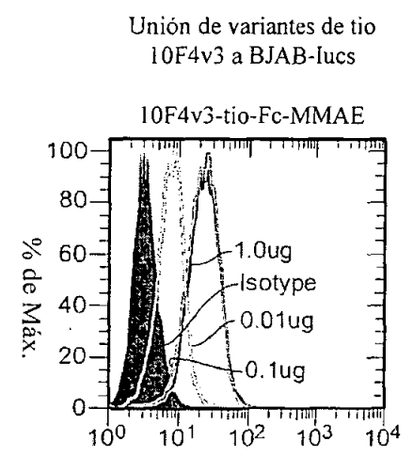


FIG. 18B

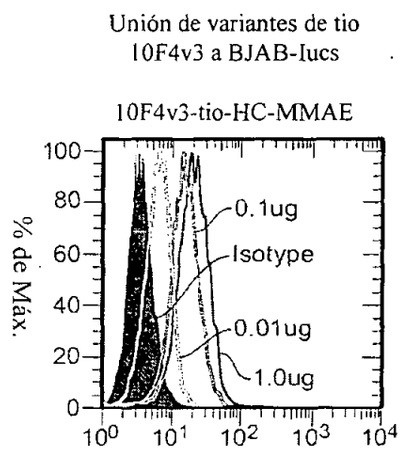


FIG. 18C

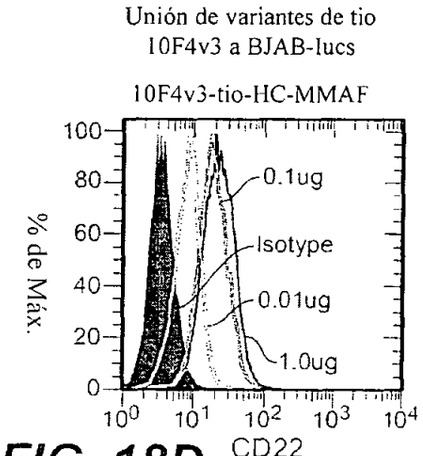


FIG. 18D

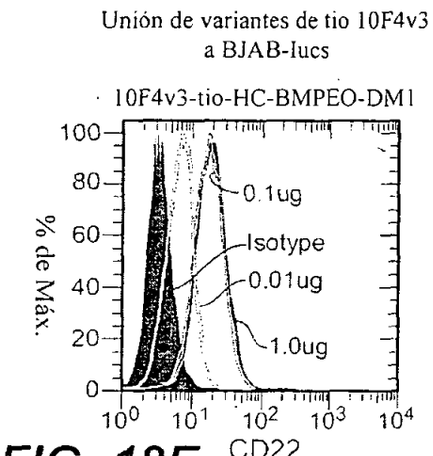


FIG. 18E

Efecto de los conjugados de thiomab anti-CD22 con fármacos administrados a ratones SCID en xenotrasplantes Granta-519

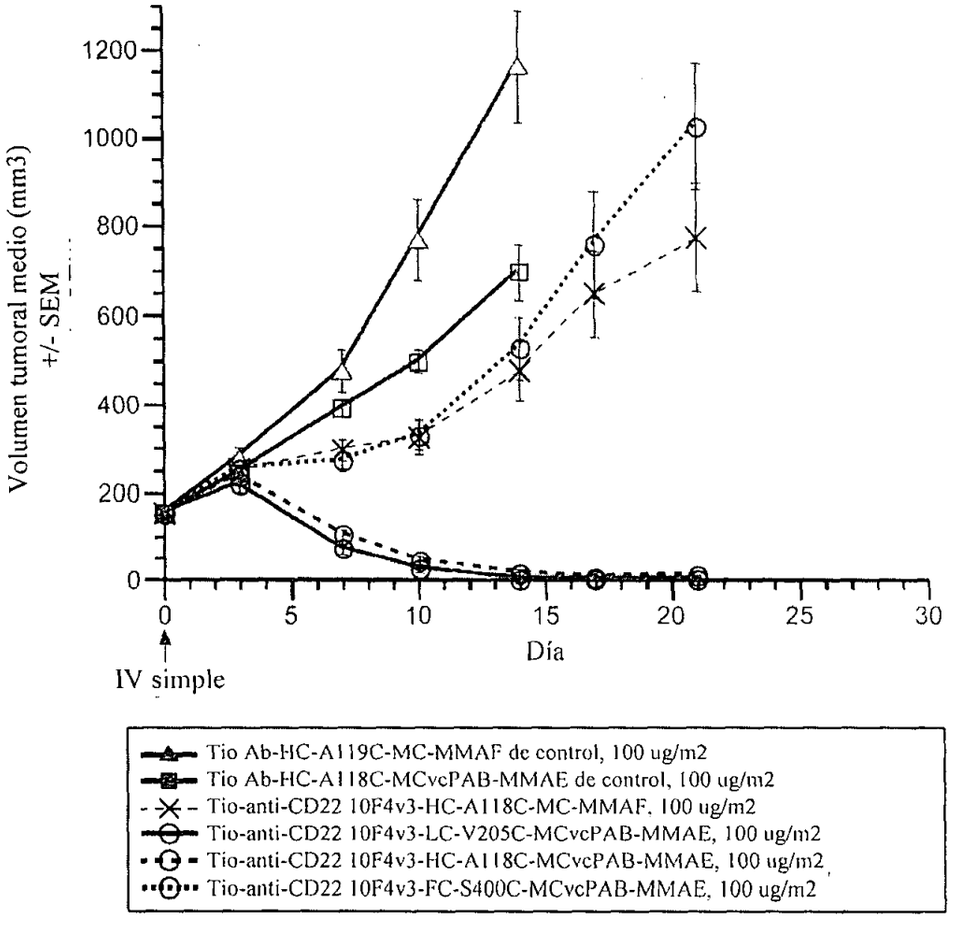


FIG. 19

Anti-CD22 tio HC(A118C) 10F4v3-BMPEO-DM1, -MCvcPAB-MMAE, y -MC-MMAF versus un xenotrasplante Granta-519 del linfoma de células del manto humano en ratones CB17 SCID

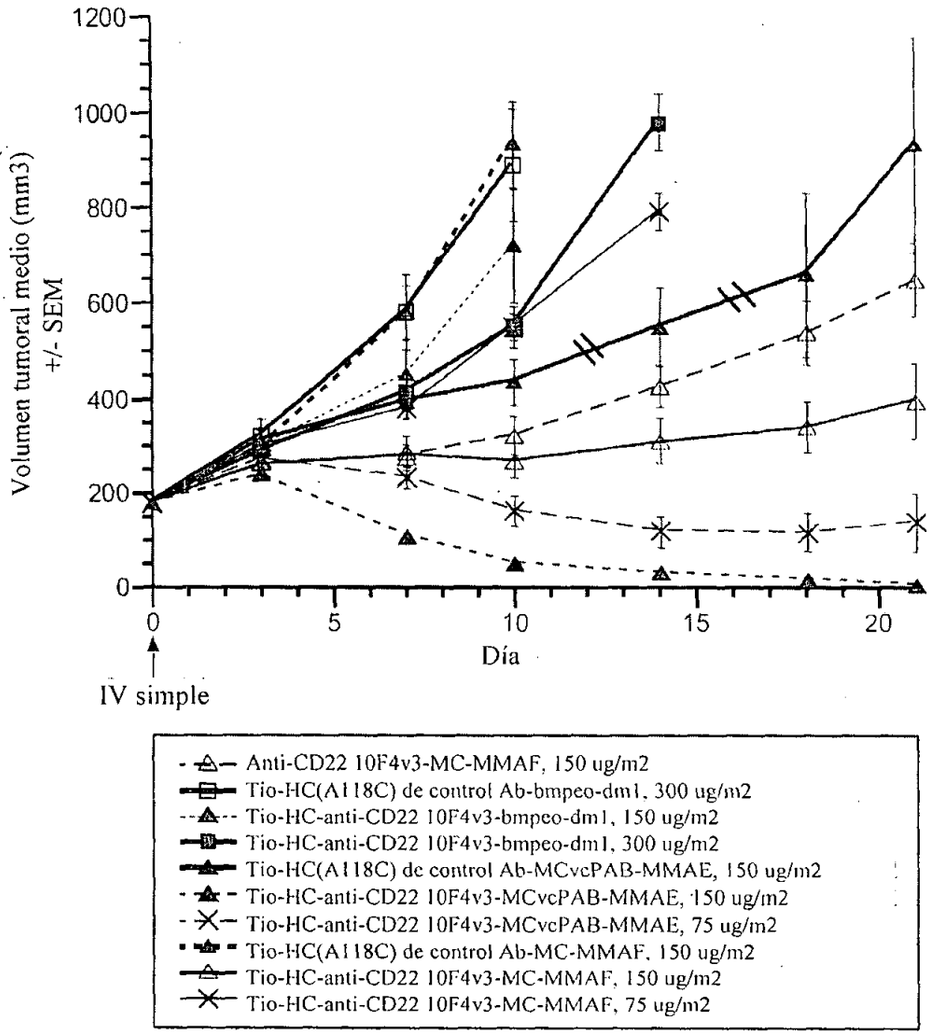


FIG. 20A

Tio-HC-A121C-anti-CD22 10F4v3-bmpeo-dm1, -MCvcPAB-MMAE, y -MC-MMAF versus un xenotrasplante DOHH2 del linfoma folicular en ratones CB17 SCID

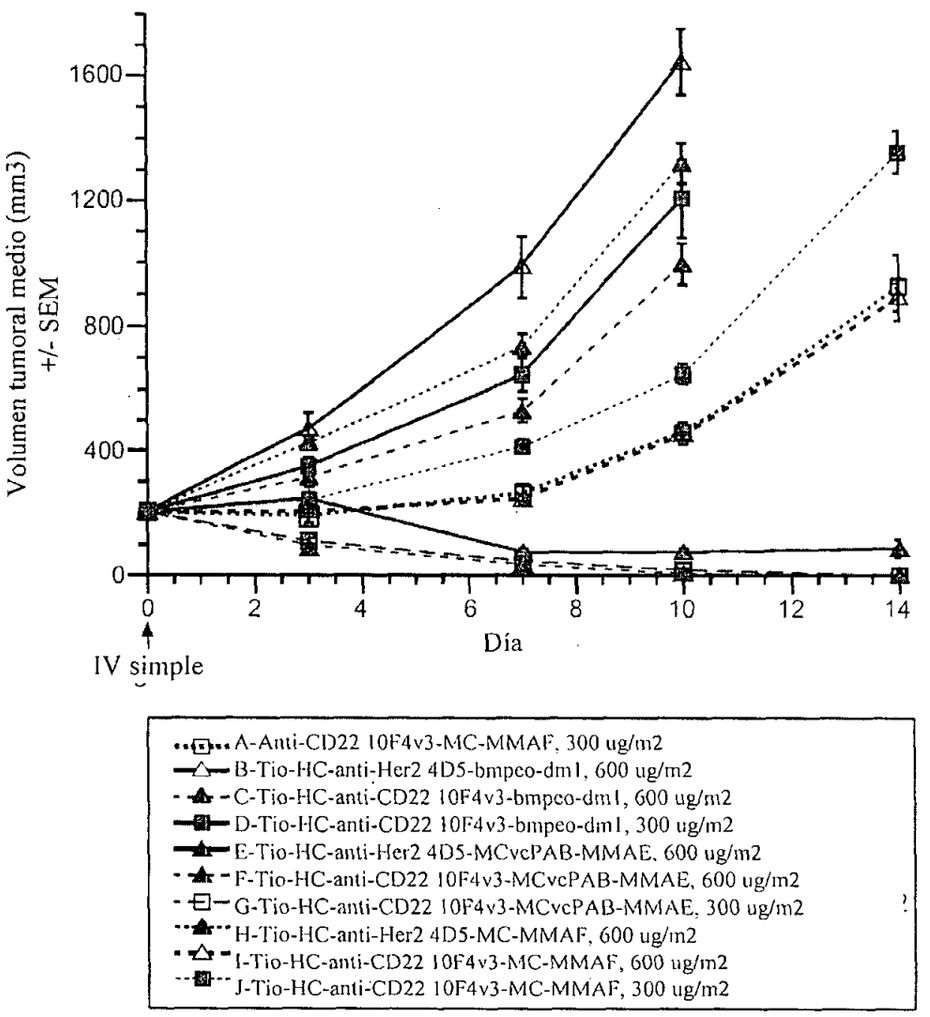


FIG. 20B

Tio-HC-A121C-anti-CD22 10F4v3-bmpeo-dm1, -MCvcPAB-MMAE, y -MC-MMAF versus un xenotrasplante DOHH2 del linfoma folicular en ratones CB17 Scid

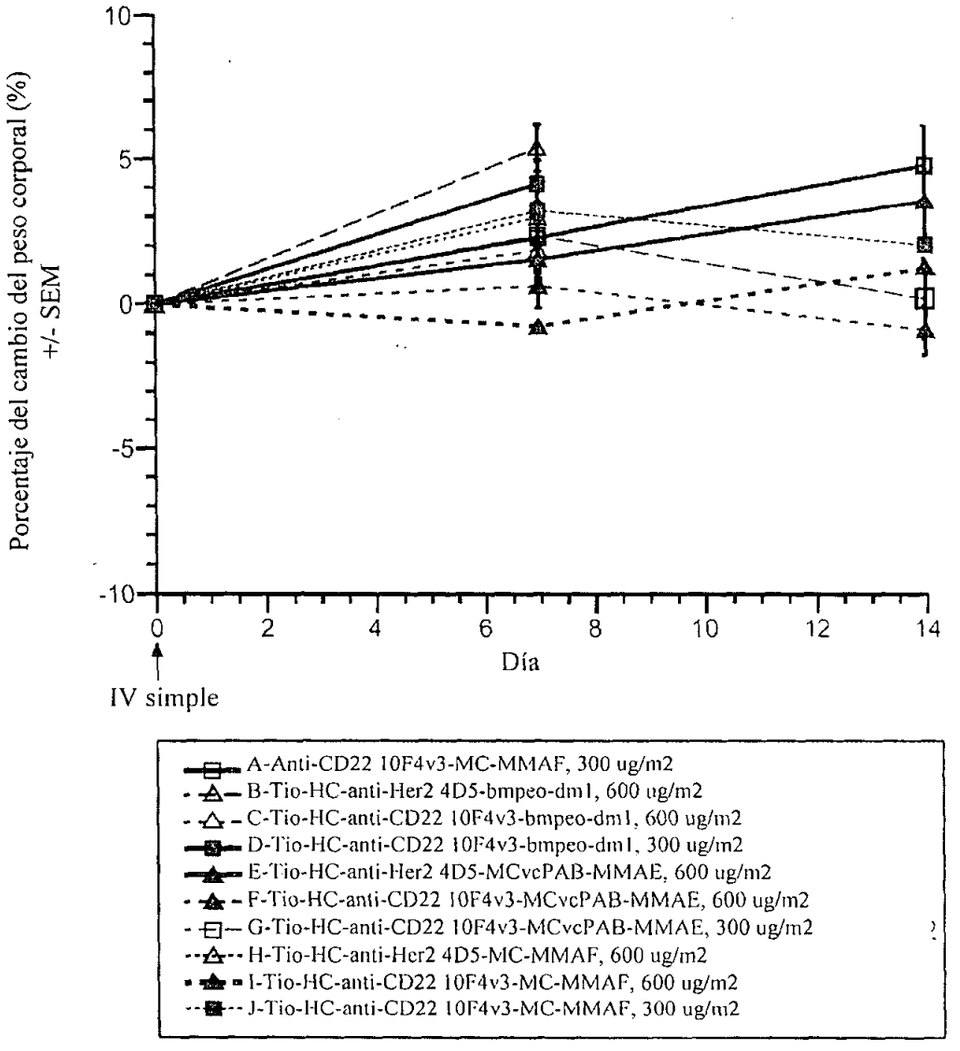


FIG. 20C

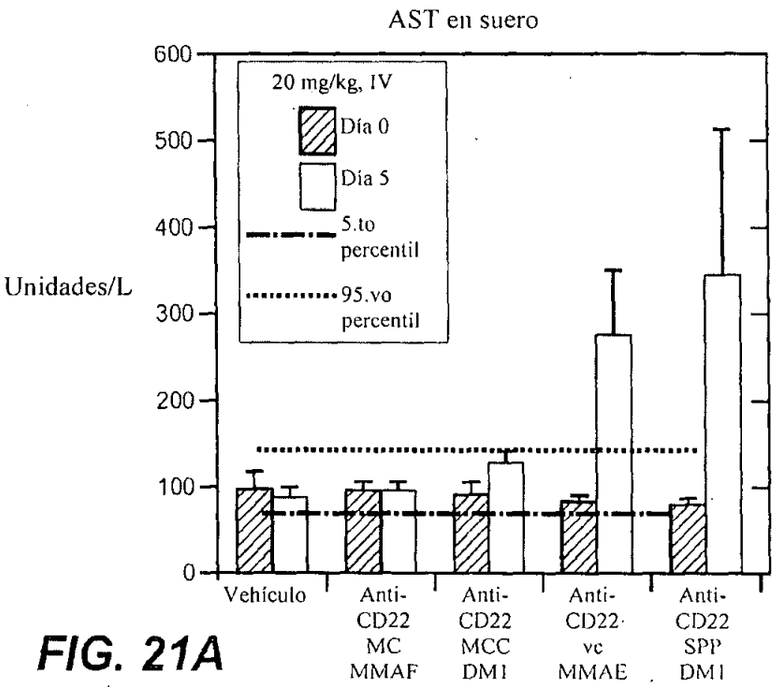


FIG. 21A

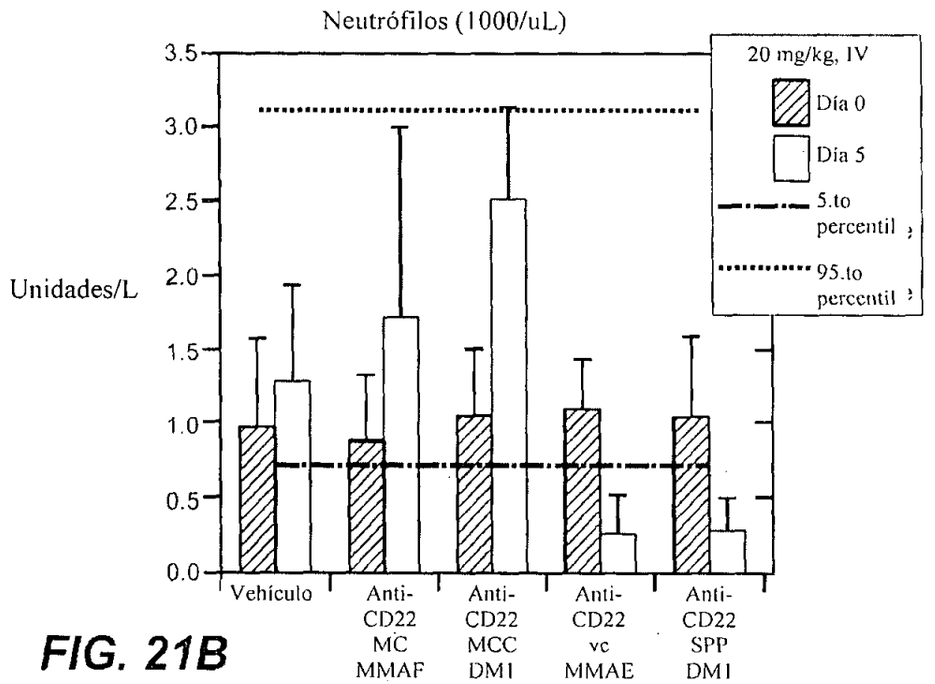


FIG. 21B

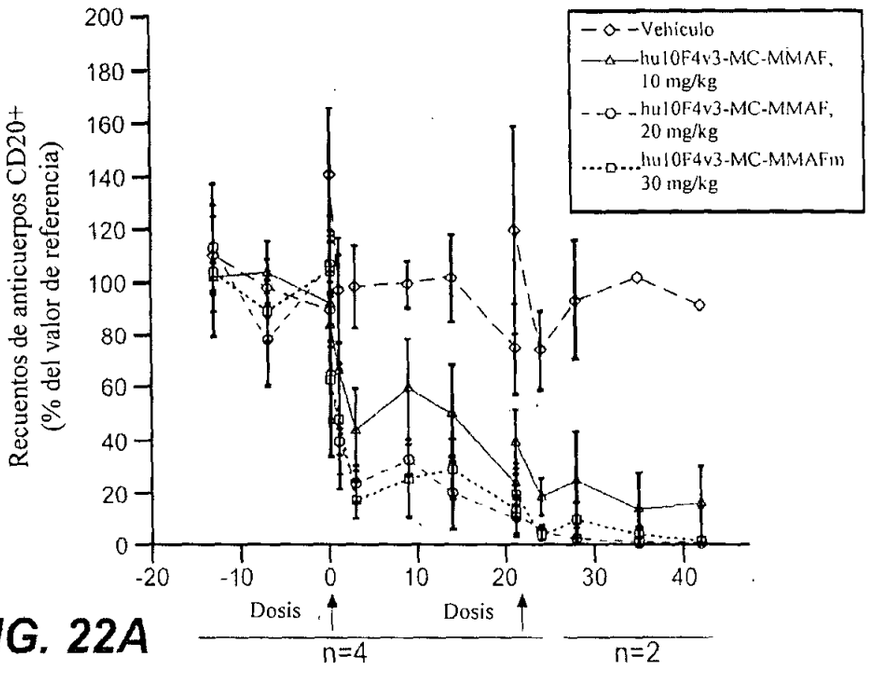


FIG. 22A

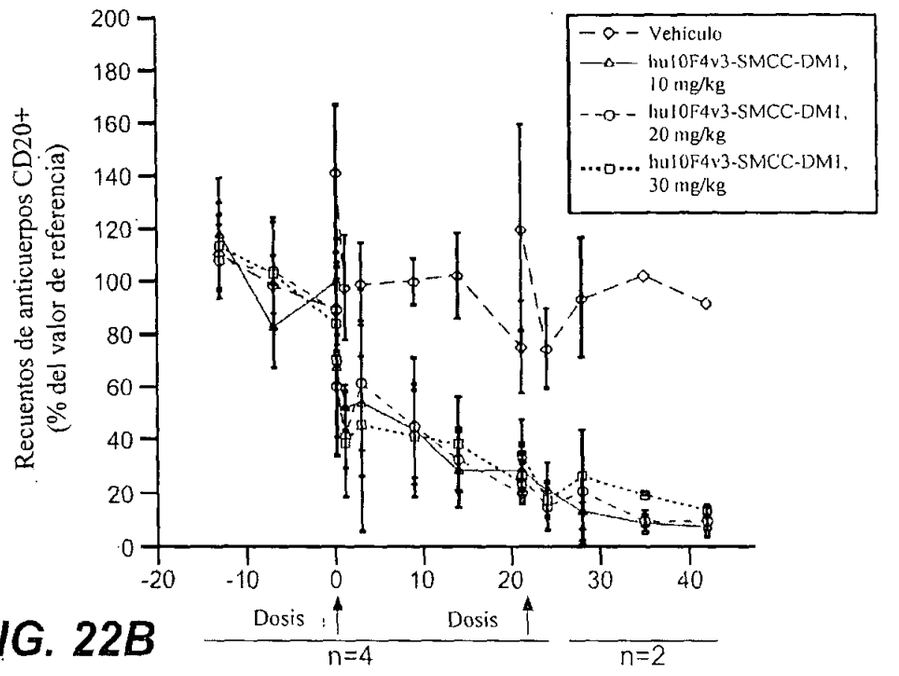


FIG. 22B

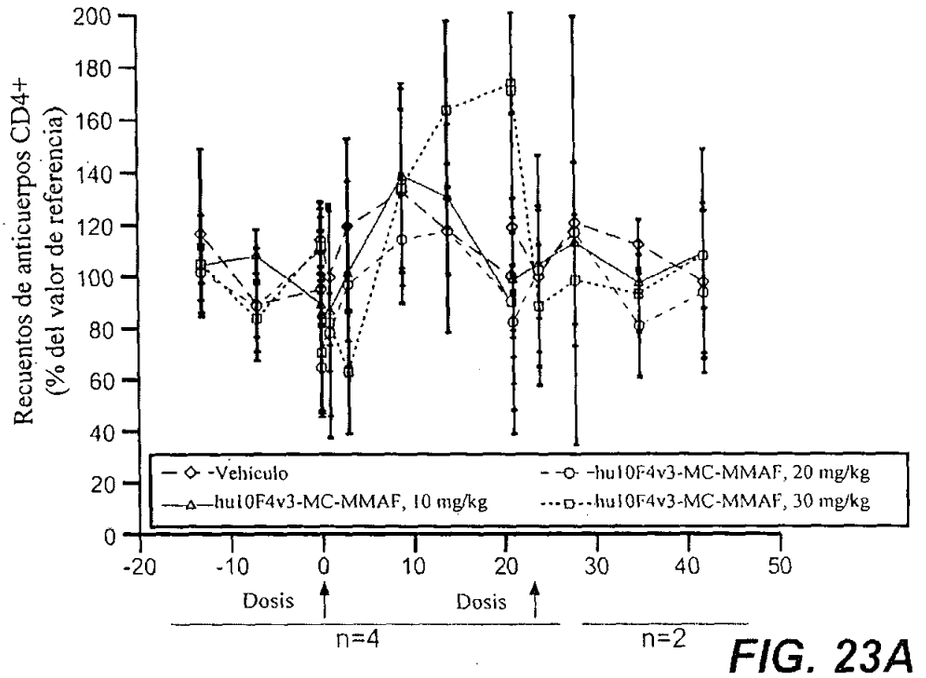


FIG. 23A

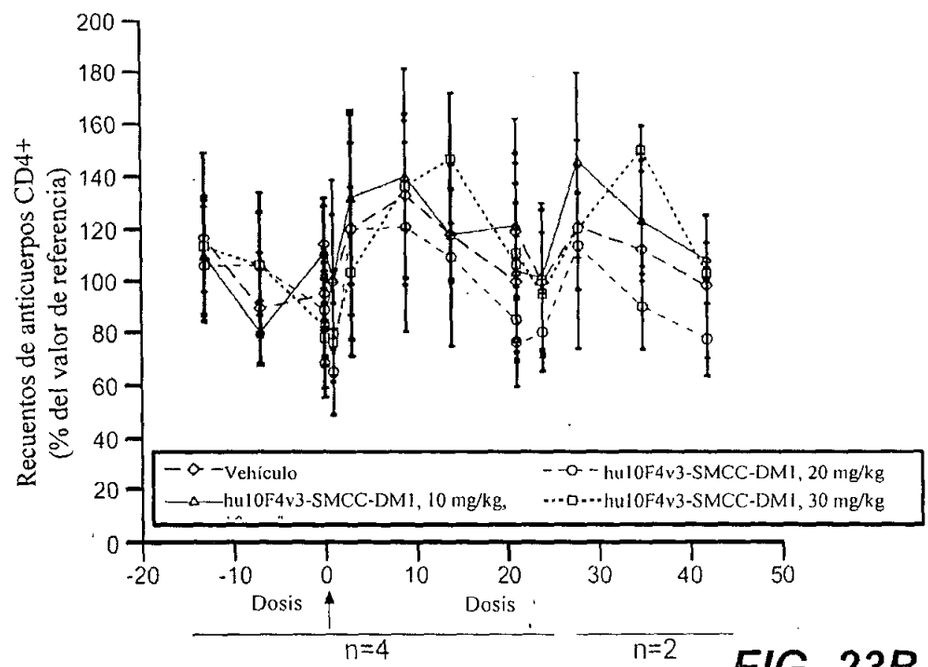


FIG. 23B

Control tonsilar del vehículo

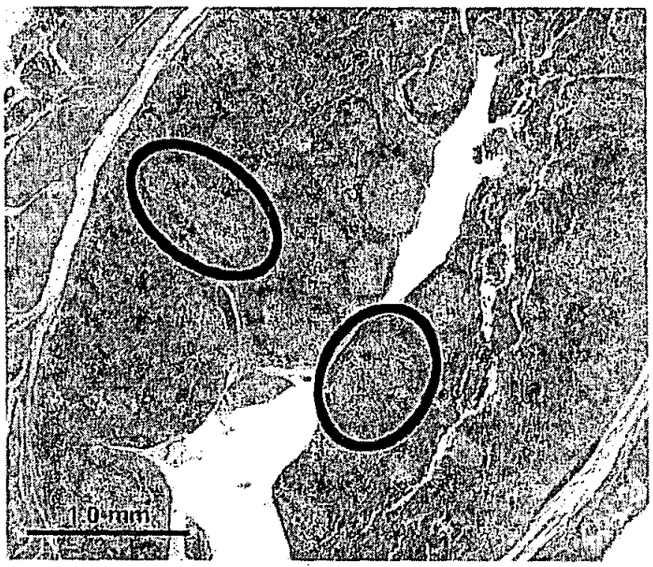


FIG. 24A

hu10F4v3-SMCC-DM1 tonsilar

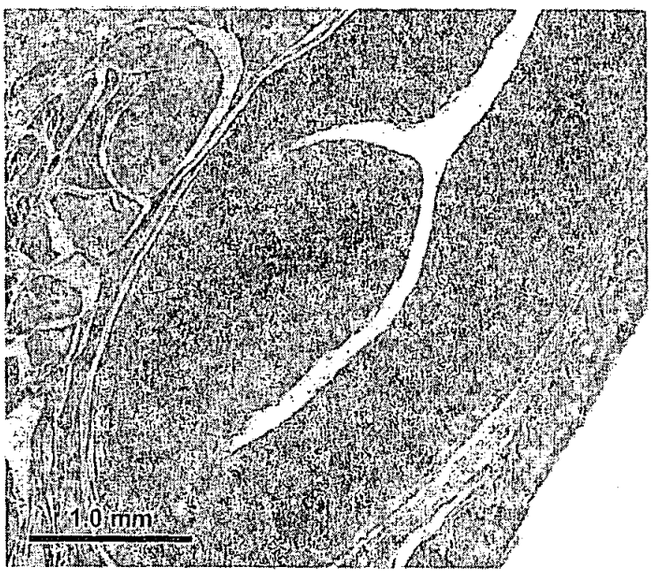


FIG. 24B

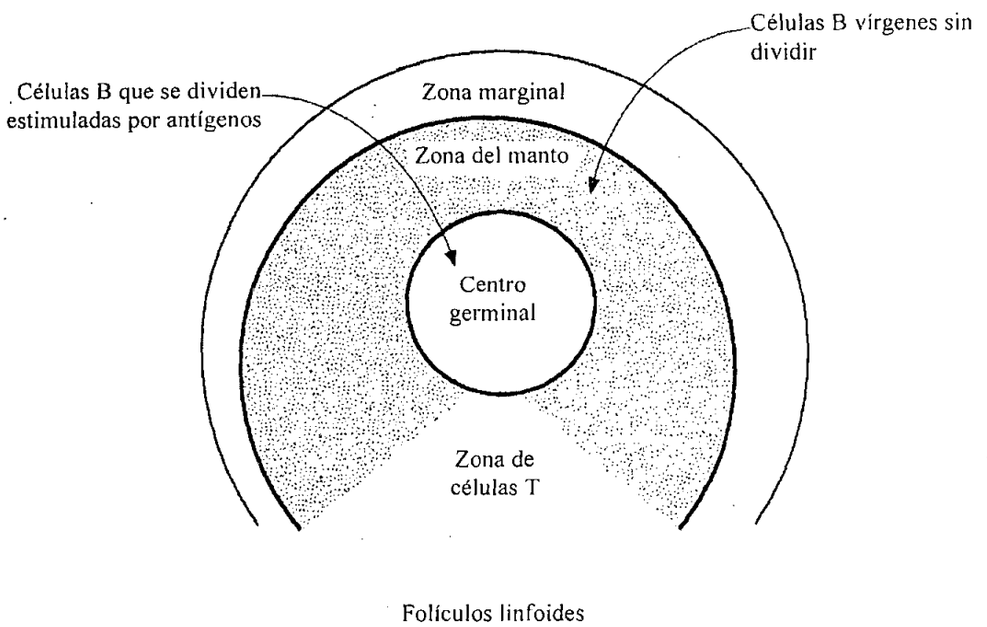
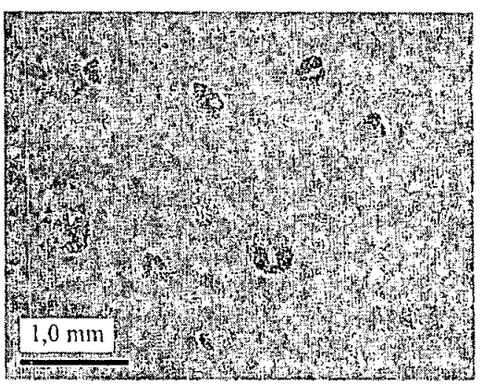

Células B vírgenes sin dividir
Células B que se dividen estimuladas por antígenos
Zona marginal
Zona del manto
Centro germinal
Zona de células T
Folículos linfoides

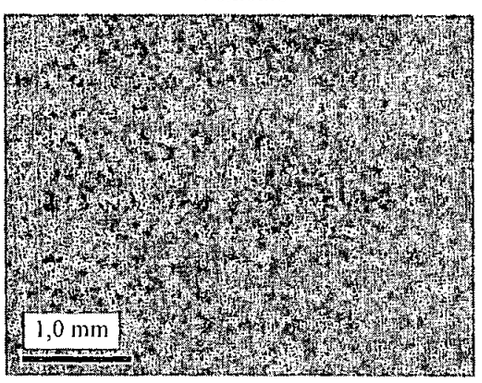
FIG. 25A

FIG. 25B

Tinción de Ki-67
(Células que se dividen)



Vehículo

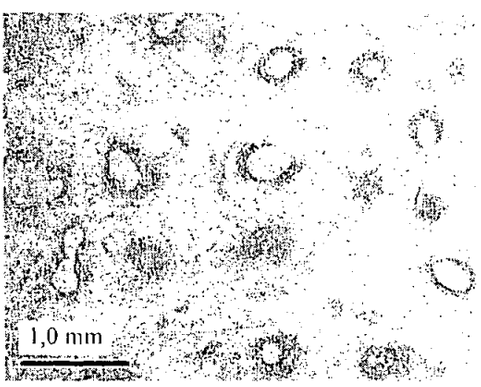


Anti-CD22-MC-MMAF
(10 mg/kg)

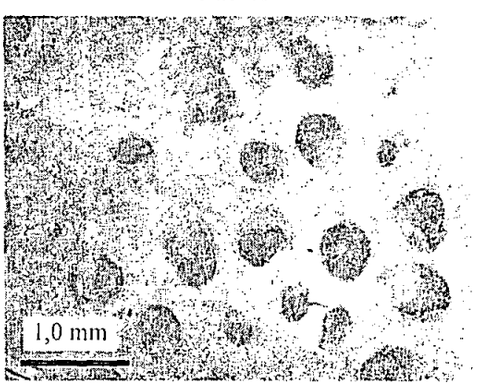
FIG. 25C

FIG. 25D

Tinción de Anti-IgD
(Células B vírgenes)



Vehículo



Anti-CD22-MC-MMAF
(10 mg/kg)

FIG. 25E

GENENLAW-#245667-v1--P2262R1_Sequence_Listing_text_in_Spanish
Listado de secuencias

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<150> US 60/911.829
<151> 2007-04-13

<150> US 60/908.941
<151> 2007-03-29

<150> US 60/809.328
<151> 2006-05-30

<160> 93

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<212> PRT
<213> Secuencia artificial

<220>
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1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 2
<211> 10
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 2
Gly Tyr Glu Phe Ser Arg Ser Trp Met Asn
5 10

<210> 3
<211> 13
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 3
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
5 10

<210> 4
<211> 18
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 4
Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Ser Gly Lys
1 5 10 15

Phe Lys Gly

<210> 5
<211> 30
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 5
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 6
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 6
Asp Gly Ser Ser Trp Asp Trp Tyr Phe Asp Val
5 10

<210> 7
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 7
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
5 10

<210> 8
<211> 23
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 8
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
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Gly Asp Arg Val Thr Ile Thr Cys

<210> 9
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 9
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 10
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 10
Arg Ser Ser Gln Ser Ile Val His Ser Val Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 11
<211> 15
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 11
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 12
<211> 7
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 12
Lys Val Ser Asn Arg Phe Ser
5

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<211> 32
<212> PRT
<213> Secuencia artificial

<220>
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<400> 13
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
20 25 30

Tyr Cys

<210> 14
<211> 9
<212> PRT
<213> Secuencia artificial

<220>
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<400> 14
Phe Gln Gly Ser Gln Phe Pro Tyr Thr
5

<210> 15
<211> 10
<212> PRT
<213> Secuencia artificial

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<400> 15
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
5 10

<210> 16
<211> 120
<212> PRT
<213> Secuencia artificial

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<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp

95 100 105

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

<210> 17
<211> 113
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 17
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg
110

<210> 18
<211> 113
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr

80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg
110

<210> 19
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 19
Arg Ser Ser Gln Ser Ile Val His Ser Ala Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 20
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 20
Arg Ser Ser Gln Ser Ile Val His Ser Gln Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 21
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 21
Arg Ser Ser Gln Ser Ile Val His Ser Ser Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 22
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<400> 22
Arg Ser Ser Gln Ser Ile Val His Ser Asp Gly Asn Thr Phe Leu

1 5 10 15

Glu

<210> 23
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 23
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Asn Thr Phe Leu
1 5 10 15

Glu

<210> 24
<211> 113
<212> PRT
<213> Secuencia artificial

<220>
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<400> 24
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Ser Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Phe Asp Tyr Trp Gly Gln
95 100 105

Gly Thr Leu Val Thr Val Ser Ser
110

<210> 25
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 25
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val

1 5 10 15

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

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

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

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

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 26
<211> 30
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 26
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

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

<210> 27
<211> 847
<212> PRT
<213> Homo sapiens

<400> 27
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr
35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser

95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val
245 250 255

Arg Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser
260 265 270

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

Leu Lys Lys Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr
290 295 300

Lys Asp Gln Ser Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val
305 310 315

Gly Pro Gly Arg Ser Glu Glu Val Phe Leu Gln Val Gln Tyr Ala
320 325 330

Pro Glu Pro Ser Thr Val Gln Ile Leu His Ser Pro Ala Val Glu
335 340 345

Gly Ser Gln Val Glu Phe Leu Cys Met Ser Leu Ala Asn Pro Leu
350 355 360

Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Met Gln Gly
365 370 375

Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu Pro Trp His
380 385 390

Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Thr Gly
395 400 405

Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro Lys

410 415 420

Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly
425 430 435

Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser
440 445 450

Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro
455 460 465

Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
470 475 480

Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser
485 490 495

Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val
500 505 510

Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val
515 520 525

Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln
530 535 540

Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln
545 550 555

Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser
560 565 570

Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp
575 580 585

Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met
590 595 600

Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr
605 610 615

Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe
620 625 630

Asp Trp Asn Asn Gln Ser Leu Pro His His Ser Gln Lys Leu Arg
635 640 645

Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln
650 655 660

Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu
665 670 675

Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val
680 685 690

Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys Gly
695 700 705

Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
710 715 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys

725 730 735

Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly
740 745 750

Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu
755 760 765

Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser
770 775 780

Ser Glu Met Gln Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr
785 790 795

Tyr Ser Ala Leu His Lys Arg Gln Val Gly Asp Tyr Glu Asn Val
800 805 810

Ile Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu
815 820 825

Ile Gln Phe Gly Val Gly Glu Arg Pro Gln Ala Gln Glu Asn Val
830 835 840

Asp Tyr Val Ile Leu Lys His
845

<210> 28
<211> 660
<212> PRT
<213> Homo sapiens

<400> 28
Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu Tyr Ala Trp Glu
1 5 10 15

Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala Leu Asp Gly
20 25 30

Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr Asn Lys
35 40 45

Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr Lys
50 55 60

Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
65 70 75

Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu
80 85 90

Asn Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu
95 100 105

Lys Trp Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe
110 115 120

Pro Pro His Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu
125 130 135

Val Thr Leu Thr Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro
140 145 150

Ile Gln Leu Gln Trp Leu Leu Glu Gly Val Pro Met Arg Gln Ala

155 160 165

Ala Val Thr Ser Thr Ser Leu Thr Ile Lys Ser Val Phe Thr Arg
170 175 180

Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser His His Gly Lys Ile
185 190 195

Val Thr Cys Gln Leu Gln Asp Ala Asp Gly Lys Phe Leu Ser Asn
200 205 210

Asp Thr Val Gln Leu Asn Val Lys His Thr Pro Lys Leu Glu Ile
215 220 225

Lys Val Thr Pro Ser Asp Ala Ile Val Arg Glu Gly Asp Ser Val
230 235 240

Thr Met Thr Cys Glu Val Ser Ser Ser Asn Pro Glu Tyr Thr Thr
245 250 255

Val Ser Trp Leu Lys Asp Gly Thr Ser Leu Lys Lys Gln Asn Thr
260 265 270

Phe Thr Leu Asn Leu Arg Glu Val Thr Lys Asp Gln Ser Gly Lys
275 280 285

Tyr Cys Cys Gln Val Ser Asn Asp Val Gly Pro Gly Arg Ser Glu
290 295 300

Glu Val Phe Leu Gln Val Gln Tyr Ala Pro Glu Pro Ser Thr Val
305 310 315

Gln Ile Leu His Ser Pro Ala Val Glu Gly Ser Gln Val Glu Phe
320 325 330

Leu Cys Met Ser Leu Ala Asn Pro Leu Pro Thr Asn Tyr Thr Trp
335 340 345

Tyr His Asn Gly Lys Glu Met Gln Gly Arg Thr Glu Glu Lys Val
350 355 360

His Ile Pro Lys Ile Leu Pro Trp His Ala Gly Thr Tyr Ser Cys
365 370 375

Val Ala Glu Asn Ile Leu Gly Thr Gly Gln Arg Gly Pro Gly Ala
380 385 390

Glu Leu Asp Val Gln Tyr Pro Pro Lys Lys Val Thr Thr Val Ile
395 400 405

Gln Asn Pro Met Pro Ile Arg Glu Gly Asp Thr Val Thr Leu Ser
410 415 420

Cys Asn Tyr Asn Ser Ser Asn Pro Ser Val Thr Arg Tyr Glu Trp
425 430 435

Lys Pro His Gly Ala Trp Glu Glu Pro Ser Leu Gly Val Leu Lys
440 445 450

Ile Gln Asn Val Gly Trp Asp Asn Thr Thr Ile Ala Cys Ala Arg
455 460 465

Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro Val Ala Leu Asn Val

470 475 480

Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys Ile Lys Pro Leu
485 490 495

Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln Cys Asp Phe
500 505 510

Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp Glu Lys Asn
515 520 525

Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe Asp Ser Ile
530 535 540

Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val Asn Asn Ser
545 550 555

Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu Val Leu Tyr
560 565 570

Ala Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly Asp Gln Val
575 580 585

Met Glu Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser Asp Ala Asn
590 595 600

Pro Pro Val Ser His Tyr Thr Trp Phe Asp Trp Asn Asn Gln Ser
605 610 615

Leu Pro His His Ser Gln Lys Leu Arg Leu Glu Pro Val Lys Val
620 625 630

Gln His Ser Gly Ala Tyr Trp Cys Gln Gly Thr Asn Ser Val Gly
635 640 645

Lys Gly Arg Ser Pro Leu Ser Thr Leu Thr Val Tyr Tyr Ser Pro
650 655 660

<210> 29
<211> 647
<212> PRT
<213> Homo sapiens

<400> 29
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr
35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser

95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Pro Pro Lys Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile
245 250 255

Arg Glu Gly Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser
260 265 270

Asn Pro Ser Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp
275 280 285

Glu Glu Pro Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp
290 295 300

Asp Asn Thr Thr Ile Ala Cys Ala Ala Cys Asn Ser Trp Cys Ser
305 310 315

Trp Ala Ser Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp
320 325 330

Val Arg Val Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly
335 340 345

Asn Ser Val Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys
350 355 360

Glu Val Gln Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys
365 370 375

Glu Ser Gln Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly
380 385 390

Ser Tyr Ser Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser
395 400 405

Lys Ala Trp Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg

410 415 420

Val Ser Met Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala
425 430 435

Thr Leu Thr Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr
440 445 450

Thr Trp Phe Asp Trp Asn Asn Gln Ser Leu Pro Tyr His Ser Gln
455 460 465

Lys Leu Arg Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr
470 475 480

Trp Cys Gln Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu
485 490 495

Ser Thr Leu Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg
500 505 510

Val Ala Val Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala
515 520 525

Ile Cys Gly Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser
530 535 540

Gln Gln Gly Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val
545 550 555

Arg Asn Lys Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His
560 565 570

Ser Leu Gly Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr
575 580 585

Thr Thr Leu Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp
590 595 600

Ala Glu Ser Ser Glu Met Gln Arg Pro Pro Pro Asp Cys Asp Asp
605 610 615

Thr Val Thr Tyr Ser Ala Leu His Lys Arg Gln Val Gly Thr Met
620 625 630

Arg Thr Ser Phe Gln Ile Phe Gln Lys Met Arg Gly Phe Ile Thr
635 640 645

Gln Ser

<210> 30
<211> 483
<212> PRT
<213> Homo sapiens

<400> 30
Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu Tyr Ala Trp Glu
1 5 10 15

Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala Leu Asp Gly
20 25 30

Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr Asn Lys

35 40 45

Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr Lys
50 55 60

Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
65 70 75

Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu
80 85 90

Asn Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu
95 100 105

Lys Trp Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe
110 115 120

Pro Pro His Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu
125 130 135

Val Thr Leu Thr Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro
140 145 150

Ile Gln Leu Gln Trp Leu Leu Glu Gly Val Pro Met Arg Gln Ala
155 160 165

Ala Val Thr Ser Thr Ser Leu Thr Ile Lys Ser Val Phe Thr Arg
170 175 180

Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser His His Gly Lys Ile
185 190 195

Val Thr Cys Gln Leu Gln Asp Ala Asp Gly Lys Phe Leu Ser Asn
200 205 210

Asp Thr Val Gln Leu Asn Val Lys His Pro Pro Lys Lys Val Thr
215 220 225

Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly Asp Thr Val
230 235 240

Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser Val Thr Arg
245 250 255

Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro Ser Leu Gly
260 265 270

Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr Thr Ile Ala
275 280 285

Cys Ala Ala Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro Val Ala
290 295 300

Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys Ile
305 310 315

Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln
320 325 330

Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp
335 340 345

Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe

350 355 360

Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val
365 370 375

Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu
380 385 390

Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly
395 400 405

Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser
410 415 420

Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe Asp Trp Asn
425 430 435

Asn Gln Ser Leu Pro Tyr His Ser Gln Lys Leu Arg Leu Glu Pro
440 445 450

Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln Gly Thr Asn
455 460 465

Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu Thr Val Tyr
470 475 480

Tyr Ser Pro

<210> 31
<211> 846
<212> PRT
<213> Pan troglodytes

<400> 31
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Glu Tyr Leu Ala
1 5 10 15

Phe Ser Asp Ser Ser Lys Trp Asn Ile Glu His Pro Gly Thr Ile
20 25 30

Tyr Ala Trp Glu Gly Ala Cys Ile Trp Val Pro Cys Thr Tyr Arg
35 40 45

Val Leu Asp Gly Ala Leu Glu Thr Phe Ile Leu Phe His Asn Pro
50 55 60

Glu Tyr Asn Gln Asn Met Ser Lys Phe Glu Gly Thr Arg Leu Tyr
65 70 75

Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Gly Gln Lys Arg Val
80 85 90

Gln Phe Leu Gly Asn Lys Ile Asn Asn Asn Cys Thr Leu Ser Ile
95 100 105

His Pro Val His Val Asn Asp Ser Gly Gln Leu Gly Leu Arg Met
110 115 120

Val Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn Val
125 130 135

Ser Glu Arg Pro Phe Pro Pro Arg Ile Gln Leu Pro Pro Lys Leu

140 145 150

Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe Ser
155 160 165

Cys Tyr Gly Tyr Gln Ile Gln Leu Gln Trp Leu Leu Glu Gly Ala
170 175 180

Pro Met Arg Gln Ala Ala Val Thr Leu Thr Ser Leu Ser Thr Lys
185 190 195

Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp Ser
200 205 210

His His Gly Lys Ile Val Thr Cys Glu Leu His Asp Val Asp Gly
215 220 225

Lys Val Leu Ser Glu Asp Thr Val Gln Leu Asn Val Lys His Thr
230 235 240

Pro Lys Leu Thr Ile Glu Val Thr Pro Asn Glu Thr Ile Val Arg
245 250 255

Lys Gly Asp Ser Val Thr Met Thr Cys Lys Val Asn Ser Ser Asn
260 265 270

Pro Glu Tyr Thr Thr Val Ser Trp Leu Lys Asp Asp Ile Pro Leu
275 280 285

Lys Glu Gln Asn Thr Leu Met Leu Thr Leu His Glu Val Thr Lys
290 295 300

Ser Gln Thr Gly Thr Tyr Cys Cys Arg Val Ser Asn Asp Val Gly
305 310 315

Pro Ala Thr Ser Glu Lys Val Phe Leu Gln Val Gln Tyr Ala Pro
320 325 330

Glu Pro Ser Arg Val Gln Ile Ser Gln Ser Pro Ala Val Glu Gly
335 340 345

Ser Glu Val Asn Phe Leu Cys Ile Ser Pro Ala Asn Pro Leu Pro
350 355 360

Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Val Gln Gly Arg
365 370 375

Thr Glu Lys Gln Phe Gln Ile Gln Lys Ile Leu Pro Trp His Ala
380 385 390

Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Ile Gly Glu
395 400 405

Arg Gly Pro Gly Thr Glu Leu Asp Val Gln Tyr Pro Pro Lys Lys
410 415 420

Val Thr Met Val Ile Glu Asn Pro Thr Pro Ile Arg Glu Gly Asp
425 430 435

Thr Val Thr Leu Ser Cys Asn Tyr Ser Ser Ser Asn Pro Ile Val
440 445 450

Asn His Tyr Glu Trp Arg Pro Arg Gly Ala Trp Glu Glu Pro Ser

455 460 465

Leu Gly Val Leu Lys Ile Gln Asn Ile Gly Trp Asn Asn Thr Ala
470 475 480

Val Ala Cys Ala Ala Cys Asn Asn Trp Cys Ser Trp Ala Ser Pro
485 490 495

Val Thr Leu Asn Val Leu Tyr Ala Pro Arg Gly Val Arg Val Arg
500 505 510

Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Leu Val Ser
515 520 525

Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe
530 535 540

Phe Trp Glu Lys Asn Gly Ser Leu Leu Gly Lys Glu Ser Gln Leu
545 550 555

Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys
560 565 570

Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr
575 580 585

Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met Ser
590 595 600

Gln Gly Asn Gln Val Met Glu Gly Lys Thr Ala Ile Leu Thr Cys
605 610 615

Glu Ser Asp Ala Asn Pro Pro Val Tyr Ser Tyr Ala Trp Phe Asp
620 625 630

Trp Asn Asn Gln Ser Leu Pro Tyr Ser Gly Arg Met Leu Arg Leu
635 640 645

Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln Gly
650 655 660

Thr Asn Arg Val Gly Lys Gly His Ser Pro Leu Ile Thr Leu Thr
665 670 675

Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val Gly
680 685 690

Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Met Cys Gly Phe
695 700 705

Lys Val Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly Leu
710 715 720

Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys
725 730 735

Val Arg Arg Thr Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys
740 745 750

Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Ala Thr Leu Arg
755 760 765

Phe Pro Glu Thr Asn Thr Pro Arg Thr Gly Asp Ala Glu Thr Ser

770 775 780

Glu Leu Gln Arg Leu Pro Pro Asp Cys Asp Asp Thr Val Thr Tyr
785 790 795

Ser Val Leu Gln Lys Arg Gln Val Gly Asp Tyr Glu Asn Val Ile
800 805 810

Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu Ile
815 820 825

Gln Phe Gly Phe Gly Glu Arg Pro Gln Ala Gln Glu Asn Val Asp
830 835 840

Tyr Val Ile Val Lys His
845

<210> 32
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 32
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Ala Thr Phe Leu
1 5 10 15

Glu

<210> 33
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 33
Arg Ser Ser Gln Ser Ile Val His Ser Ile Gly Gln Thr Phe Leu
1 5 10 15

Glu

<210> 34
<211> 120
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 34
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

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

Arg Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Arg

35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

<210> 35
<211> 112
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 35
Asp Ile Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
1 5 10 15

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Leu Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gly
95 100 105

Gly Thr Lys Val Glu Ile Lys
110

<210> 36
<211> 847
<212> PRT
<213> Homo sapiens

<400> 36
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr
1 5 10 15

Leu Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu
20 25 30

Thr Leu Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr

35 40 45

Tyr Arg Ala Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His
50 55 60

Asn Pro Glu Tyr Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg
65 70 75

Leu Tyr Glu Ser Thr Lys Asp Gly Lys Val Pro Ser Glu Gln Lys
80 85 90

Arg Val Gln Phe Leu Gly Asp Lys Asn Lys Asn Cys Thr Leu Ser
95 100 105

Ile His Pro Val His Leu Asn Asp Ser Gly Gln Leu Gly Leu Arg
110 115 120

Met Glu Ser Lys Thr Glu Lys Trp Met Glu Arg Ile His Leu Asn
125 130 135

Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Leu Pro Pro Glu
140 145 150

Ile Gln Glu Ser Gln Glu Val Thr Leu Thr Cys Leu Leu Asn Phe
155 160 165

Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp Leu Leu Glu Gly
170 175 180

Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser Leu Thr Ile
185 190 195

Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro Gln Trp
200 205 210

Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala Asp
215 220 225

Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
230 235 240

Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val
245 250 255

Arg Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser
260 265 270

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

Leu Lys Lys Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr
290 295 300

Lys Asp Gln Ser Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val
305 310 315

Gly Pro Gly Arg Ser Glu Glu Val Phe Leu Gln Val Gln Tyr Ala
320 325 330

Pro Glu Pro Ser Thr Val Gln Ile Leu His Ser Pro Ala Val Glu
335 340 345

Gly Ser Gln Val Glu Phe Leu Cys Met Ser Leu Ala Asn Pro Leu

350 355 360

Pro Thr Asn Tyr Thr Trp Tyr His Asn Gly Lys Glu Met Gln Gly
365 370 375

Arg Thr Glu Glu Lys Val His Ile Pro Lys Ile Leu Pro Trp His
380 385 390

Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn Ile Leu Gly Thr Gly
395 400 405

Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln Tyr Pro Pro Lys
410 415 420

Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile Arg Glu Gly
425 430 435

Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn Pro Ser
440 445 450

Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu Pro
455 460 465

Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
470 475 480

Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser
485 490 495

Pro Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val
500 505 510

Arg Lys Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val
515 520 525

Ser Leu Gln Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln
530 535 540

Phe Phe Trp Glu Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln
545 550 555

Leu Asn Phe Asp Ser Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser
560 565 570

Cys Trp Val Asn Asn Ser Ile Gly Gln Thr Ala Ser Lys Ala Trp
575 580 585

Thr Leu Glu Val Leu Tyr Ala Pro Arg Arg Leu Arg Val Ser Met
590 595 600

Ser Pro Gly Asp Gln Val Met Glu Gly Lys Ser Ala Thr Leu Thr
605 610 615

Cys Glu Ser Asp Ala Asn Pro Pro Val Ser His Tyr Thr Trp Phe
620 625 630

Asp Trp Asn Asn Gln Ser Leu Pro His His Ser Gln Lys Leu Arg
635 640 645

Leu Glu Pro Val Lys Val Gln His Ser Gly Ala Tyr Trp Cys Gln
650 655 660

Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu Ser Thr Leu

665 670 675

Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val Ala Val
680 685 690

Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys Gly
695 700 705

Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
710 715 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys
725 730 735

Lys Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly
740 745 750

Cys Tyr Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu
755 760 765

Arg Phe Pro Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser
770 775 780

Ser Glu Met Gln Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr
785 790 795

Tyr Ser Ala Leu His Lys Arg Gln Val Gly Asp Tyr Glu Asn Val
800 805 810

Ile Pro Asp Phe Pro Glu Asp Glu Gly Ile His Tyr Ser Glu Leu
815 820 825

Ile Gln Phe Gly Val Gly Glu Arg Pro Gln Ala Gln Glu Asn Val
830 835 840

Asp Tyr Val Ile Leu Lys His
845

<210> 37
<211> 42
<212> PRT
<213> Homo sapiens

<400> 37
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
1 5 10 15

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

Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys
35 40

<210> 38
<211> 218
<212> PRT
<213> Homo sapiens

<400> 38
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val

20 25 30

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
35 40 45

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
50 55 60

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
65 70 75

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
80 85 90

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
95 100 105

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
110 115 120

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
125 130 135

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
140 145 150

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
155 160 165

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
170 175 180

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
185 190 195

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
200 205 210

Ser Leu Ser Leu Ser Pro Gly Lys
215

<210> 39
<211> 217
<212> PRT
<213> Homo sapiens

<400> 39
Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
1 5 10 15

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
20 25 30

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
35 40 45

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
50 55 60

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
65 70 75

Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

80 85 90

Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr
95 100 105

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
110 115 120

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
125 130 135

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
140 145 150

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
155 160 165

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
170 175 180

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
185 190 195

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
200 205 210

Leu Ser Leu Ser Pro Gly Lys
215

<210> 40
<211> 217
<212> PRT
<213> Homo sapiens

<400> 40
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe
35 40 45

Lys Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
50 55 60

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val
65 70 75

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
80 85 90

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
95 100 105

Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
110 115 120

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
125 130 135

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

140 145 150

Trp Glu Ser Ser Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr Pro
155 160 165

Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
170 175 180

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys
185 190 195

Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser
200 205 210

Leu Ser Leu Ser Pro Gly Lys
215

<210> 41
<211> 218
<212> PRT
<213> Homo sapiens

<400> 41
Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
1 5 10 15

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
20 25 30

Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln
35 40 45

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
50 55 60

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
65 70 75

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
80 85 90

Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
95 100 105

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
110 115 120

Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser
125 130 135

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
140 145 150

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
155 160 165

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg
170 175 180

Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
185 190 195

Cys Ser Val Met His Glu Ala Leu His Asn His Thr Tyr Gln Lys

200 205 210

Ser Leu Ser Leu Ser Leu Gly Lys
215

<210> 42
<211> 30
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 42
Cys Asp Lys Thr His Thr Gly Gly Gly Ser Gln Arg Leu Met Glu
1 5 10 15

Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp Asp Phe
20 25 30

<210> 43
<211> 20
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 43
Gln Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu
1 5 10 15

Trp Glu Asp Asp Phe
20

<210> 44
<211> 20
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 44
Gln Arg Leu Ile Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu
1 5 10 15

Trp Glu Asp Asp Phe
20

<210> 45
<211> 18
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 45
Arg Leu Ile Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
1 5 10 15

Glu Asp Asp

<210> 46
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 46
Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp
5 10

<210> 47
<211> 14
<212> PRT
<213> Homo sapiens

<400> 47
Trp Val Arg Arg Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
5 10

<210> 48
<211> 32
<212> PRT
<213> Homo sapiens

<400> 48
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

Ala Arg

<210> 49
<211> 23
<212> PRT
<213> Homo sapiens

<400> 49
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro
1 5 10 15

Gly Glu Pro Ala Ser Ile Ser Cys
20

<210> 50
<211> 25
<212> PRT
<213> Homo sapiens

<400> 50
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser
20 25

<210> 51

<211> 13
<212> PRT
<213> Homo sapiens

<400> 51
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
5 10

<210> 52
<211> 31
<212> PRT
<213> Homo sapiens

<400> 52
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

Ala

<210> 53
<211> 30
<212> PRT
<213> Homo sapiens

<400> 53
Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr Met
1 5 10 15

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

<210> 54
<211> 30
<212> PRT
<213> Homo sapiens

<400> 54
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15

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

<210> 55
<211> 14
<212> PRT
<213> Homo sapiens

<400> 55
Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Gly
5 10

<210> 56
<211> 32
<212> PRT
<213> Homo sapiens

<400> 56
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu

1 5 10 15

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

Ala Arg

<210> 57
<211> 25
<212> PRT
<213> Homo sapiens

<400> 57
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser
1 5 10 15

Gln Thr Leu Ser Leu Thr Cys Thr Val Ser
20 25

<210> 58
<211> 13
<212> PRT
<213> Homo sapiens

<400> 58
Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
5 10

<210> 59
<211> 31
<212> PRT
<213> Homo sapiens

<400> 59
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
1 5 10 15

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

Ala

<210> 60
<211> 30
<212> PRT
<213> Homo sapiens

<400> 60
Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
1 5 10 15

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

<210> 61
<211> 30
<212> PRT
<213> Homo sapiens

<400> 61
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

1 5 10 15

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

<210> 62
<211> 14
<212> PRT
<213> Homo sapiens

<400> 62
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
5 10

<210> 63
<211> 32
<212> PRT
<213> Homo sapiens

<400> 63
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala Arg

<210> 64
<211> 25
<212> PRT
<213> Homo sapiens

<400> 64
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser
20 25

<210> 65
<211> 13
<212> PRT
<213> Homo sapiens

<400> 65
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
5 10

<210> 66
<211> 31
<212> PRT
<213> Homo sapiens

<400> 66
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala

<210> 67
<211> 30
<212> PRT
<213> Homo sapiens

<400> 67
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 68
<211> 30
<212> PRT
<213> Homo sapiens

<400> 68
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

<210> 69
<211> 32
<212> PRT
<213> Homo sapiens

<400> 69
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ser Arg

<210> 70
<211> 31
<212> PRT
<213> Homo sapiens

<400> 70
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ser

<210> 71
<211> 32
<212> PRT
<213> Homo sapiens

<400> 71
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu

1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala Arg

<210> 72
<211> 31
<212> PRT
<213> Homo sapiens

<400> 72
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

Ala

<210> 73
<211> 30
<212> PRT
<213> Homo sapiens

<400> 73
Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu
1 5 10 15

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
20 25 30

<210> 74
<211> 23
<212> PRT
<213> Homo sapiens

<400> 74
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys
20

<210> 75
<211> 15
<212> PRT
<213> Homo sapiens

<400> 75
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 76
<211> 32
<212> PRT
<213> Homo sapiens

<400> 76
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe

1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
20 25 30

Tyr Cys

<210> 77
<211> 10
<212> PRT
<213> Homo sapiens

<400> 77
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
5 10

<210> 78
<211> 14
<212> PRT
<213> Homo sapiens

<400> 78
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
5 10

<210> 79
<211> 15
<212> PRT
<213> Homo sapiens

<400> 79
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> 80
<211> 32
<212> PRT
<213> Homo sapiens

<400> 80
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

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

Tyr Cys

<210> 81
<211> 23
<212> PRT
<213> Homo sapiens

<400> 81
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
1 5 10 15

Gly Glu Arg Ala Thr Leu Ser Cys
20

<210> 82

<211> 15
<212> PRT
<213> Homo sapiens

<400> 82
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 83
<211> 32
<212> PRT
<213> Homo sapiens

<400> 83
Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr
20 25 30

Tyr Cys

<210> 84
<211> 23
<212> PRT
<213> Homo sapiens

<400> 84
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu
1 5 10 15

Gly Glu Arg Ala Thr Ile Asn Cys
20

<210> 85
<211> 15
<212> PRT
<213> Homo sapiens

<400> 85
Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 86
<211> 32
<212> PRT
<213> Homo sapiens

<400> 86
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
1 5 10 15

Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr
20 25 30

Tyr Cys

<210> 87
<211> 219
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 87
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
110 115 120

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
125 130 135

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
140 145 150

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
155 160 165

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
170 175 180

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
185 190 195

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
200 205 210

Thr Lys Ser Phe Asn Arg Gly Glu Cys
215

<210> 88
<211> 450
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 88
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Glu Phe Ser

20 25 30

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
320 325 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln

335 340 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

<210> 89
<211> 120
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 89
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

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

Arg Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Arg
35 40 45

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

<210> 90
<211> 112
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 90
Asp Ile Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu
1 5 10 15

Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Asn Gly Asn Thr Phe Leu Glu Trp Tyr Leu Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gly
95 100 105

Gly Thr Lys Val Glu Ile Lys
110

<210> 91
<211> 219
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 91
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Ile Val
20 25 30

His Ser Val Gly Asn Thr Phe Leu Glu Trp Tyr Gln Gln Lys Pro
35 40 45

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

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
65 70 75

Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
80 85 90

Tyr Tyr Cys Phe Gln Gly Ser Gln Phe Pro Tyr Thr Phe Gly Gln
95 100 105

Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
110 115 120

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
125 130 135

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

140 145 150

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
155 160 165

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
170 175 180

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
185 190 195

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Cys
200 205 210

Thr Lys Ser Phe Asn Arg Gly Glu Cys
215

<210> 92
<211> 450
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 92
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Cys Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser

185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
320 325 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
335 340 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

<210> 93
<211> 450
<212> PRT
<213> Secuencia artificial

<220>
<223> secuencia sintetizada

<400> 93
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

1 5 10 15

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

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

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr
50 55 60

Ser Gly Lys Phe Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser
65 70 75

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Gly Ser Ser Trp Asp Trp
95 100 105

Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
125 130 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
140 145 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
155 160 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
170 175 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
185 190 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
200 205 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
215 220 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
230 235 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
260 265 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
305 310 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala

320 325 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
335 340 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
350 355 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
365 370 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
380 385 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Cys Asp Gly
395 400 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
410 415 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
425 430 435

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445 450

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1 and Administrative Instructions, Section 422)

To:

CONLEY, Deirdre L.
c/o GENENTECH, INC.
1 DNA Way MS 49
South San Francisco, California 94080
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
12 March 2008 (12.03.2008)

Applicant's or agent's file reference
P2262R1

IMPORTANT NOTIFICATION

International application No.
PCT/US2007/069889

International filing date *(day/month/year)*
29 May 2007 (29.05.2007)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address
WU, Yan
846 Andromeda Lane
Foster City, California 94404
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address
WU, Yan
1160 Blythe Street
Foster City, California 94404
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☐ the International Searching Authority
☐ the International Preliminary Examining Authority
☒ the designated Offices concerned
☐ the elected Offices concerned
☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Blanc Veronique
e-mail Veronique.Blanc@wipo.int
Telephone No. +41 22 338 96 66

701

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/US2007/069889	29.05.2007	30.05.2006

International Patent Classification (IPC) or both national classification and IPC
INV. A61K47/48 C07K16/28

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 D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Sommerfeld, Teresa Telephone No. +49 89 2399-7197

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. 142, 143, 145,146,148-152,154,221,223-225

because:

☒ the said international application, or the said claims Nos. 142,143,145,146,148-152,154,221,223-225 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	1-238
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-238
Industrial applicability (IA)	Yes:	Claims	1-141,144,147,153,155-220,222,226-238
	No:	Claims	

2. Citations and explanations

see separate sheet

The following documents have been cited in the search report:

D1: WO 2006/042240 A (WYETH CORP [US]; IYER ANAND [US]; DUNUSSI-JOANNOPOULOS KYRIAKI [US]) 20 April 2006 (2006-04-20)

D2: WO 03/072736 A (UNIV DUKE [US]; TEDDER THOMAS F [US]) 4 September 2003 (2003-09-04)

D3: WO 03/093320 A (CELLTECH R & D LTD [GB]; POPPLEWELL ANDREW GEORGE [GB]; TICKLE SIMON P) 13 November 2003 (2003-11-13)

D4: WO 2005/113003 A (GENENTECH INC [US]; CHAN ANDREW C [US]; GONG QIAN [US]; MARTIN FLAVIUS) 1 December 2005 (2005-12-01)

D5: LEONARD JOHN P ET AL: "Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 AUG 2004, vol. 10, no. 16, 15 August 2004 (2004-08-15), pages 5327-5334, XP002459865 ISSN: 1078-0432

D6: DIJOSEPH J F ET AL: "ANTIBODY-TARGETED CHEMOTHERAPY WITH CMC-544: A CD22-TARGETED IMMUNOCONJUGATE OF CALICHEAMICIN FOR THE TREATMENT OF B-LYMPHOID MALIGNANCIES" BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, US, vol. 103, no. 5, March 2004 (2004-03), pages 1807-1814, XP008060153 ISSN: 0006-4971

D7: WO 2005/117986 A (GENENTECH INC [US]; EBENS JR ALLEN J [US]; JACOBSON FREDERIC S [US]; P) 15 December 2005 (2005-12-15)

Introduction

The present application discloses anti-CD22 antibodies and immunoconjugates thereof, and their use for treatment of B-cell proliferative disorders, such as lymphomas and leukemias.

Section III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 142, 143, 145, 146, 148-152, 154, 221 and 223-225 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).

Section V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 discloses anti-CD22 antibodies. Seq. ID. No. 28 of D1, corresponding to the light chain of an anti-CD22 humanized antibody (CDR-grafted) is 92.24% identical to Seq. ID. No. 87 of the present application. Document D1 also discloses conjugation to cytotoxic drugs such as maytansinoids and linkers (paragraphs 79-81).

2. Document D2 also discloses anti-CD22 antibodies. Seq. ID. No. 29 of D2, corresponding to an anti-human CD22 antibody light chain, comprises Seq. ID. No. 12 of the application. Document D2 also discloses treatment of B-cell malignancies (paragraph 002) and immunoconjugates with e.g. maytansinoid (paragraph 96).

3. Similarly, also document D3 discloses anti-CD22 antibodies and their use for treatment of malignant lymphomas or leukemias (claim 38; page 15 lines 11-12). Seq. ID. No. 28 of D3 (anti-CD22 light chain) is 92.24% identical to Seq. ID. No. 87 of the application.

4. Any of the abovementioned documents can be considered the closest prior art for the subject-matter of claim 1. The difference to the present application is that the specific antibodies of claim 1 are not disclosed. The technical problem can thus be formulated as the provision of further anti-CD22 antibodies.
   It appears that the mere provision of further antibodies against anti-CD22, such as those of claim 1, would be trivial for the skilled person since CD22 was well known, other anti-CD22 antibodies were available, and their relevance in therapy of B-cell lymphoproliferative disorders was also known.
   Claim 1 can thus be considered to lack inventive step (Art. 33(3) PCT).

5. Further claims merely add features which appear to be routine variations to the

subject-matter of claim 1.

Claim 2-238 are thus considered to lack inventive step as well (Art. 33(3) PCT).

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

Bitte beachten Sie, dass angeführte Nichtpatentliteratur (wie z. B. wissenschaftliche oder technische Dokumente) je nach geltendem Recht dem Urheberrechtsschutz und/oder anderen Schutzarten für schriftliche Werke unterliegen könnte. Die Vervielfältigung urheberrechtlich geschützter Texte, ihre Verwendung in anderen elektronischen oder gedruckten Publikationen und ihre Weitergabe an Dritte ist ohne ausdrückliche Zustimmung des Rechtsinhabers nicht gestattet.

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XS CPRTENFRDE

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

CONLEY, Deirdre L.
c/o GENENTECH, INC.
1 DNA Way MS 49
South San Francisco, California 94080
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 29 August 2007 (29.08.2007)	
Applicant's or agent's file reference P2262R1	IMPORTANT NOTIFICATION
International application No. PCT/US2007/069889	International filing date *(day/month/year)* 29 May 2007 (29.05.2007)
International publication date *(day/month/year)* **Not yet published**	Priority date *(day/month/year)* 30 May 2006 (30.05.2006)
Applicant GENENTECH, INC. et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)***An asterisk (*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
30 May 2006 (30.05.2006)	60/809,328	US	21 August 2007 (21.08.2007)
29 March 2007 (29.03.2007)	60/908,941	US	21 August 2007 (21.08.2007)
13 April 2007 (13.04.2007)	60/911,829	US	21 August 2007 (21.08.2007)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Athina Nickitas-Etienne
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Telephone No. +41 22 338 74 04

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PCT REQUEST

Original (for **SUBMISSION**)

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT-SAFE [EASY mode] Version 3.51.018.193 MT/FOP 20070401/0.20.5.9
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	**Applicant's or agent's file reference**	P2262R1
I	**Title of Invention**	ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR
II	**Applicant**	
II-1	This person is	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	GENENTECH, INC.
II-5	Address	1 DNA Way South San Francisco, California 94080 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	650-225-1000
II-9	Facsimile No.	650-952-9881
III-1	**Applicant and/or inventor**	
III-1-1	This person is	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	EBENS, JR., Allen J.
III-1-5	Address	1932 Arroyo Avenue San Carlos, California 94070 United States of America
III-1-6	State of nationality	US
III-1-7	State of residence	US

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PCT REQUEST

Original (for **SUBMISSION**)

III-2	**Applicant and/or inventor**	
III-2-1	This person is	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	GRAY, Alane M.
III-2-5	Address	122 Kestrel Court Brisbane, California 94005 United States of America
III-2-6	State of nationality	US
III-2-7	State of residence	US
III-3	**Applicant and/or inventor**	
III-3-1	This person is	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	LIANG, Wei-Ching
III-3-5	Address	840 Sea Spray Lane, #112 Foster City, California 94404 United States of America
III-3-6	State of nationality	CN
III-3-7	State of residence	US
III-4	**Applicant and/or inventor**	
III-4-1	This person is	applicant and inventor
III-4-2	Applicant for	US only
III-4-4	Name (LAST, First)	WU, Yan
III-4-5	Address	846 Andromeda Lane Foster City, California 94404 United States of America
III-4-6	State of nationality	US
III-4-7	State of residence	US
III-5	**Applicant and/or inventor**	
III-5-1	This person is	applicant and inventor
III-5-2	Applicant for	US only
III-5-4	Name (LAST, First)	YU, Shang-Fan
III-5-5	Address	1103 Sandalwood Lane Milpitas, California 95035 United States of America
III-5-6	State of nationality	CN
III-5-7	State of residence	US

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PCT REQUEST

Original (for SUBMISSION)

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	CONLEY, Deirdre L.
IV-1-2	Address	c/o GENENTECH, INC. 1 DNA Way MS 49 South San Francisco, California 94080 United States of America
IV-1-3	Telephone No.	650-225-2066
IV-1-4	Facsimile No.	650-952-9881
IV-1-5	e-mail	conley.deirdre@gene.com
IV-1-6	Agent's registration No.	36487
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	AGARWAL,Atulya(40887); ANDRUS,Alex(44509); BARNES,Elizabeth M.(35059); BARRY,Anna L.(51436); COBURN,Cara M.(46631); CUI,Steven X.(44637); ELLIOTT,Jennifer(50584); EVANS,David(L0035); HASAK,Janet E.(28616); JOHNSTON,Sean A.(35910); KALINOWSKI,Grant(48314); KRESNAK,Mark T.(42767); KUBINEC,Jeffrey S.(36575); LEE,Wendy M.(40378); MARSCHANG,Diane L.(35600); NAIK,Paul(49075); PASQUALONE,Danielle(43847); PLEASURE,Irene(45506); SCHWARTZ,Timothy R.(32171); SHERMAN,Daniel(53586); SHIN,Elinor K.(43117); SVOBODA,Craig G.(39044); TAN,Lee K.(39447); WANG,Min(51303); YEUNG,Bonny(53811)
V	DESIGNATIONS	
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**	

PCT REQUEST

Original (for **SUBMISSION**)

VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	30 May 2006 (30.05.2006)
VI-1-2	Number	60/809,328
VI-1-3	Country	US
VI-2	**Priority claim of earlier national application**	
VI-2-1	Filing date	29 March 2007 (29.03.2007)
VI-2-2	Number	60/908,941
VI-2-3	Country	US
VI-3	**Priority claim of earlier national application**	
VI-3-1	Filing date	13 April 2007 (13.04.2007)
VI-3-2	Number	60/911,829
VI-3-3	Country	US
VI-4	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1, VI-2, VI-3
VI-5	**Incorporation by reference :** where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)

PCT REQUEST

Original (for **SUBMISSION**)

VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	**Check list**	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	6	✓
IX-2	Description (excluding sequence listing part)	255	-
IX-3	Claims	21	-
IX-4	Abstract	1	✓
IX-5	Drawings	31	-
IX-7a	Sub-total number of sheets	314	
IX-6a	Sequence listing part of description	0	-
IX-7	TOTAL	314	
	Accompanying Items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	✓	-
IX-16(A)	Sequence listing in electronic form		
IX-16(A)-(i)	copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	-	1 txt document
IX-17	PCT-SAFE physical media	-	✓
IX-19	**Figure of the drawings which should accompany the abstract**	NONE	
IX-20	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	/Deirdre L. CONLEY Reg. No. 36,487/	
X-1-1	Name (LAST, First)	CONLEY, Deirdre L.	
X-1-2	Name of signatory		
X-1-3	Capacity		

PCT REQUEST

Original (for **SUBMISSION**)

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAY 04, 2007

PTAS

500271109A

500271109A

DEIRDRE L. CONLEY
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT SERVICES BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 05/04/2007 REEL/FRAME: 019251/0812
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: PR2262-2

ASSIGNOR:
EBENS, ALLEN J., JR. DOC DATE: 05/04/2007

ASSIGNOR:
GRAY, ALANE M. DOC DATE: 05/04/2007

ASSIGNOR:
LIANG, WEI-CHING DOC DATE: 05/03/2007

ASSIGNOR:
WU, YAN DOC DATE: 05/02/2007

ASSIGNOR:
YU, SHANG-FAN DOC DATE: 05/03/2007

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019251/0812 PAGE 2

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080

SERIAL NUMBER: 60911829 FILING DATE: 04/13/2007
PATENT NUMBER: ISSUE DATE:
TITLE: ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

727

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

05/04/2007
500271109

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Allen J. Ebens Jr.	05/04/2007
Alane M. Gray	05/04/2007
Wei-Ching Liang	05/03/2007
Yan Wu	05/02/2007
Shang-Fan Yu	05/03/2007

RECEIVING PARTY DATA

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

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	60911829

CH $40.00 60911829

CORRESPONDENCE DATA

Fax Number: (650)952-9881
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 650/225-2066
Email: cmricks@gene.com
Correspondent Name: Deirdre L. Conley
Address Line 1: 1 DNA Way
Address Line 4: South San Francisco, CALIFORNIA 94080

ATTORNEY DOCKET NUMBER:	PR2262-2
NAME OF SUBMITTER:	Deirdre L. Conley

728

Total Attachments: 2
source=PR2282-2Assignment#page1.tif
source=PR2282-2Assignment#page2.tif

729

Docket No. PR2262-2

ASSIGNMENT

WHEREAS, Allen J. Ebens, Jr., a citizen of the United States, residing at 1932 Arroyo Avenue, San Carlos, CA 94070, and Alane M. Gray, a citizen of the United States, residing at 122 Kestrel Court, Brisbane, CA 94005, and Wei-Ching Liang, a citizen of Taiwan, residing at 840 Sea Spray Lane #112, Foster City, CA 94404, and Yan Wu, a citizen of the United States, residing at 846 Andromeda Lane, Foster City, CA 94404, and Shang-Fan Yu, a citizen of Taiwan, residing at 1103 Sandalwood Lane, Milpitas, CA 95035 (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

for which a provisional application Serial No. 60/911,829 (Docket No. PR2262-2) has been filed by them on April 13, 2007; 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;

South San Francisco
Dated: 5/4/2007

Allen Ebens

South San Francisco
Dated: 5.4.2007

Alane M. Gray

South San Francisco
Dated: 5/3/2007

Wei-Ching Liang

South San Francisco
Dated: 5/2/2007

Yan Wu

South San Francisco
Dated: 5/3/2007

Shang-Fan Yu

731

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

NOVEMBER 01, 2006

PTAS

500172665A

500172665A

DEIRDRE L. CONLEY
1 DNA WAY
M/S49
SO. SAN FRANCISCO, CA 94080-4990

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT SERVICES BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 11/01/2006

REEL/FRAME: 018464/0050
NUMBER OF PAGES: 7

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: PR2262

ASSIGNOR:
EBENS, ALLEN — DOC DATE: 10/25/2006

ASSIGNOR:
GRAY, ALANE — DOC DATE: 10/30/2006

ASSIGNOR:
LIANG, WEI-CHING — DOC DATE: 10/23/2006

ASSIGNOR:
WU, YAN — DOC DATE: 10/23/2006

732

018464/0050 PAGE 2

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY
SO. SAN FRANCISCO, CALIFORNIA

94080-4990

SERIAL NUMBER: 06080932
PATENT NUMBER: 4326888
TITLE: ANTI-RUST COMPOSITION

FILING DATE: 10/01/1979
ISSUE DATE: 04/27/1982

ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

733

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

11/01/2006
500172665

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Allen Ebens	10/25/2006
Alane Gray	10/30/2006
Wei-Ching Liang	10/23/2006
Yan Wu	10/23/2006

RECEIVING PARTY DATA

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

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	06080932

CH --$40.00 --- 06080932

CORRESPONDENCE DATA

Fax Number: (650)952-9881
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 650-225-2066
Email: hebert.barbara@gene.com
Correspondent Name: Deirdre L. Conley
Address Line 1: 1 DNA Way
Address Line 2: M/S49
Address Line 4: So. San Francisco, CALIFORNIA 94080-4990

ATTORNEY DOCKET NUMBER:	PR2282
NAME OF SUBMITTER:	Deirdre L. Conley

734

Total Attachments: 5
source=PR2262_Assignment#page1.tif
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source=PR2262_Assignment#page5.tif

735

Docket No. PR2262

ASSIGNMENT

WHEREAS, Allen Ebens, a citizen of US, residing at 1932 Arroyo Ave, San Carlos, CA 94070, and Alane Gray, a citizen of US, residing at 122 Kestrel Court, Brisbane, CA 94005, and Wei-Ching Liang, a citizen of TW, residing at 840 Sea Spray Lane #112, Foster City, CA 94404, and Yan Wu, a citizen of US, residing at 846 Andromeda Lane, Foster City, CA 94404, and , a citizen of , residing at (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

for which a provisional application Serial No. 60/809,328 (Docket No. PR2262) has been filed by them on May 30, 2006; 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.

736

Application No. 60/809,328 Docket No. PR2262

IN WITNESS WHEREOF we undersign as follows;

South San Francisco

Dated: 10/25/2006

Allen Ebens

South San Francisco

Dated: _____

Alane Gray

South San Francisco

Dated: _____

Wei-Ching Liang

South San Francisco

Dated: _____

Yan Wu

Application No. 60/809,328 Docket No. PR2262

IN WITNESS WHEREOF we undersign as follows;

South San Francisco

Dated: _____

_____

Allen Ebens

South San Francisco

Dated: 10·30·2006

Alane Gray

South San Francisco

Dated: _____

_____

Wei-Ching Liang

South San Francisco

Dated: _____

_____

Yan Wu

738

Application No. 60/809,328 Docket No. PR2262

IN WITNESS WHEREOF we undersign as follows;

South San Francisco

Dated: _____

_____
Allen Ebens

South San Francisco

Dated: _____

_____
Alane Gray

South San Francisco

Dated: 10/23/06

Wei-Ching Liang

South San Francisco

Dated: _____

_____
Yan Wu

Application No. 60/809,328 Docket No. PR2262

IN WITNESS WHEREOF we undersign as follows;

South San Francisco

Dated: _____

_____

Allen Ebens

South San Francisco

Dated: _____

_____

Alane Gray

South San Francisco

Dated: _____

_____

Wei-Ching Liang

South San Francisco

Dated: 10/23/2006.

Yan Wu

DLC/cc 7/8

PR2262-1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAY 01, 2007

PTAS

500268575A

500268575A

DEIRDRE L. CONLEY
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94070

RECEIVED
MAY 0 8 2007
GENENTECH, INC.
LEGAL DEPT.

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT SERVICES BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 05/01/2007

REEL/FRAME: 019233/0730
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: PR2262-1

ASSIGNOR:
EBENS, ALLEN J. — DOC DATE: 04/27/2007

ASSIGNOR:
GRAY, ALANE M. — DOC DATE: 04/27/2007

ASSIGNOR:
LIANG, WEI-CHING — DOC DATE: 04/25/2007

ASSIGNOR:
WU, YAN — DOC DATE: 04/27/2007

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY

SOUTH SAN FRANCISCO, CALIFORNIA

94080

741

SERIAL NUMBER: 60908941 FILING DATE: 03/29/2007
PATENT NUMBER: ISSUE DATE:
TITLE: ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

05/01/2007
500268575

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Allen J. Ebens	04/27/2007
Alane M. Gray	04/27/2007
Wei-Ching Liang	04/25/2007
Yan Wu	04/27/2007

RECEIVING PARTY DATA

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

PROPERTY NUMBERS Total: 1

Property Type	Number
Application Number:	60908941

CH $40.00 60908941

CORRESPONDENCE DATA

Fax Number: (650)982-9881
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 650/225-2066
Email: cmricks@gene.com
Correspondent Name: Deirdre L. Conley
Address Line 1: 1 DNA Way
Address Line 4: South San Francisco, CALIFORNIA 94070

ATTORNEY DOCKET NUMBER:	PR2262-1
NAME OF SUBMITTER:	Deirdre L. Conley

Total Attachments: 2
source=PR2262-1Assignment#page1.tif

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744

Docket No. PR2262-1

ASSIGNMENT

WHEREAS, Allen J. Ebens, Jr., a citizen of the United States, residing at 1932 Arroyo Avenue, San Carlos, CA 94070, and Alane M. Gray, a citizen of the United States, residing at 122 Kestrel Court, Brisbane, CA 94005, and Wei-Ching Liang, a citizen of Taiwan, residing at 840 Sea Spray Lane #112, Foster City, CA 94404, and Yan Wu, a citizen of the United States, residing at 846 Andromeda Lane, Foster City, CA 94404, (hereinafter "ASSIGNORS"), have invented a new and useful invention in

ANTIBODIES AND IMMUNOCONJUGATES AND USES THEREFOR

for which a provisional application Serial No. 60/908,941 (Docket No. PR2262-1) has been filed by them on March 29, 2007; 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;

South San Francisco
Dated: 4/27/07

Allen Ebens

South San Francisco
Dated: 4.27.07

Alane M. Gray

South San Francisco
Dated: 04/25/07

Wei-Ching Liang

South San Francisco
Dated: 4.27.07

Yan Wu

746

State of California

SECRETARY OF STATE

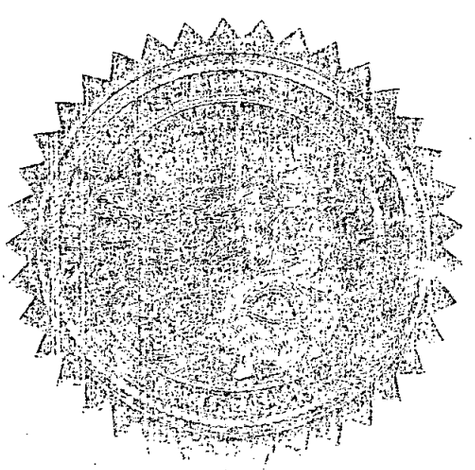
APOSTILLE

(Convention de La Haye du 5 octobre 1961)

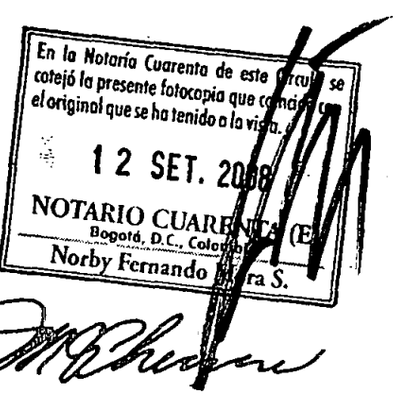
1. *Country: United States of America*
   This public document
2. *has been signed by AIDA MICLAT GUIAM*
3. *acting in the capacity of Notary Public, State of California*
4. *bears the seal/stamp of AIDA MICLAT GUIAM, Notary Public, State of California*

CERTIFIED

5. *At Sacramento, California*
6. *the 15th day of December 2005*
7. *by Deputy Secretary of State, State of California*
8. *No. 267083*
9. *Seal/Stamp:*



10. Signature



Secretary of State

BY

NP-24 A (REV. 4-05)

OSP 05 90772

74

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California
County of San Mateo } ss.

On December 6, 2005 (Date) before me, Aida Miclat Guiam (Name and Title of Officer (e.g., "Jane Doe, Notary Public"))

personally appeared Timothy R. Schwartz (Name(s) of Signer(s))

☐ personally known to me
☒ proved to me on the basis of satisfactory evidence

to be the person~~(s)~~ whose name~~(s)~~ is/~~are~~ subscribed to the within instrument and acknowledged to me that he/~~she/they~~ executed the same in his/~~her/their~~ authorized capacity~~(ies)~~, and that by his/~~her/their~~ signature~~(s)~~ on the instrument the person~~(s)~~, or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal.

Signature of Notary Public

AIDA MICLAT GUIAM
Commission # 1455662
Notary Public - California
San Mateo County
My Comm. Expires Dec 9, 2007

OPTIONAL

Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

Description of Attached Document

Title or Type of Document: _____

Document Date: _____ Number of Pages: _____

Signer(s) Other Than Named Above: _____

Capacity(ies) Claimed by Signer

Signer's Name: _____

☐ Individual
☐ Corporate Officer — Title(s): _____
☐ Partner — ☐ Limited ☐ General
☐ Attorney-in-Fact
☐ Trustee
☐ Guardian or Conservator
☐ Other: _____

Signer Is Representing: _____

RIGHT THUMBPRINT OF SIGNER
Top of thumb here

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En la Notaría Cuarenta de este Círculo s
cotejó la presente fotocopia que coincide co
el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

748

Brigard & Castro

BOGOTA - COLOMBIA

PODER

Yo (nosotros)

domiciliado(s) en

Nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **BEATRIZ JARAMILLO** y/o **CARLOS H. PALACIO EASTMAN** y/o **HECTOR GOMEZ MEJIA**, de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(s) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, aclaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeren conducentes al resguardo de mis (nuestros intereses), con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer, y la de sustituirlo en todo o en parte y revocar sustituciones. Asimismo nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **BEATRIZ JARAMILLO** y/o **CARLOS H. PALACIO EASTMAN** y/o **HECTOR GOMEZ MEJIA**,, domiciliado(s) en la Carrera 7 No. 16-56 Piso 9, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales.

Otorgado y firmado en

hoy de de

Firma

Nombre

Cargo

En la Notaría Cuarenta de este Círculo cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA
Bogotá, D.C., Colombia
Norby Fernando Mora S.

POWER OF ATTORNEY

I (We) **Genentech, Inc.**
1 DNA Way

domiciled in **South San Francisco, California 94080-4990 USA**

Hereby appoints **JUAN PABLO CADENA SARMIENTO** and/or **BEATRIZ JARAMILLO** and/or **CARLOS H. PALACIO EASTMAN** and/or **HECTOR GOMEZ MEJIA**, of Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names and emblems, registration of copyright and contracts related thereto, certificates of obtainers of vegetables varieties, as well as extensions, renewals and recordal of assignments and changes of name and domicile related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or explotation of licenses, action of unfair competition; criminal proceedings, actions for payment of damages: claims or observations on patent applications, models, industrial designs and utility models; in the exercise of preventive measures and in other actions or similar proceedings.

To that effect they hereby empowered to file applications, formulate descriptions, amendments, protests, declarations, oppositions, cancellations, nullities, appeals and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights granted or in the process of granting; to receive all kinds of documents and valuables, giving the respective release of receipt, and, in general, to take before said authorities the necessary steps to such effect, and any measures that they may deem pertinent to the safeguard of my (our) interest(s) with all the other necessary legal powers.

This power includes the faculty to ratify or confirm desist, settle and compromise on my (our) behalf, and to substitute this power in whole or in part and to revoke substitutions. At the same time I (We) hereby appoints **JUAN PABLO CADENA SARMIENTO** and/or **BEATRIZ JARAMILLO** and/or **CARLOS H. PALACIO EASTMAN** and/or **HECTOR GOMEZ MEJIA**, domiciled in Carrera 7 No. 16-56, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

Granted and signed in **South San Francisco, California USA**

this 16th day of December 2005

Signature

Name Timothy R. Schwartz, Ph.D.

Title Section Patent Counsel
Authorized Corporate Signatory
Genentech, Inc.

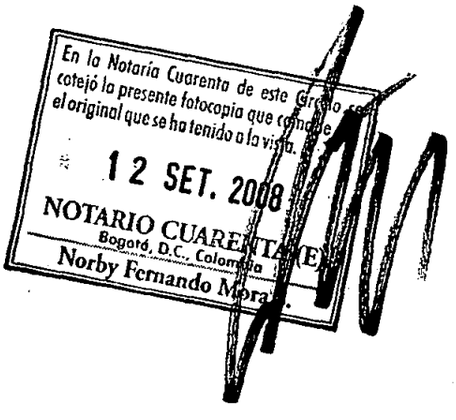
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AUTHORIZATION

Genentech, Inc. hereby authorizes any of Janet E. Hasak, Mark T. Kresnak, Jeffrey S. Kubinec and Timothy R. Schwartz, for so long as they remain employees of Genentech, Inc., to execute, on its behalf, any submissions associated with the filing, prosecution, granting, and extension and maintenance of grant of patent and trademark applications in the U.S. and foreign countries, contracts relating to biological deposits with the American Type Culture Collection, and assignments to publishers of copyrights covering works authored by Genentech employees.

August 27, 2002
Dated

Sean Johnston
Sean A. Johnston
Vice President, Intellectual Property
Genentech, Inc.



750

TRADUCCION AL ESPAÑOL del Documento de Poder otorgado por **Genentech, Inc.** domiciliada en **1 DNA Way, South San Francisco, California 94080-4990, Estados Unidos de América**.

ESTADO DE CALIFORNIA
SECRETARIA DE ESTADO

APOSTILLA
(Convención de La Haya del 5 de Octubre de 1961)

1 País: Estados Unidos de América
Esta documento público
2. ha sido firmado por AIDA MICLAT GUIAM
3. actuando en capacidad de Notario Público, Estado de California
4. lleva el sello/timbre de AIDA MICLAT GUIAM, Notario Público Estado de California

CERTIFICADO

5. en Sacramento, California
6. el 15 de diciembre de 2005
7. por el Secretario de Estado Delegado, Estado de California
8. No. 267083
9. Sello/Timbre

10. Firma

(Firma)
Secretario de Estado

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12 SET. 2008
NOTARIO CUARENTA
Bogotá, D.C., Colombia
Norby Fernando Mora S.

RECONOCIMIENTO TODO-PROPOSITO DE CALIFORNIA

Estado de California)
} ss.
Condado de San Mateo)

El 6 de diciembre de 2005, ante mi, AIDA MICLAT GUIAM, apareció personalmente Thimoty R. Schwartz

☐ a quien conozco personalmente

☒ quien demostró ante mi en base a evidencia satisfactoria ser la persona cuyo nombre esta suscrito en el instrumento y quien reconoció ante mi que ejecutó el mismo en su capacidad autorizada, y que por su firma en el instrumento la persona o la entidad a nombre de la cual la persona actuó, ejecutó el instrumento.

Hay un sello que dice:
AIDA MICLAT GUIAM
COMISIÓN # 14556612
Notario Público-California
Condado de San Mateo
Mi comisión expira Dic. 9 de 2007

Atestigua mi firma y sello oficial,

AIDA MICLAT GUIAM

----------**OPCIONAL**----------

Aunque la información en esta sección no es requerida por la ley, puede probar ser valiosa para las personas que se basan en el documento y puede prevenir la remoción y readhesión fraudulenta de esta forma a otro documento

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Nombre del firmante:_____

☐ Individuo
☐ Funcionario Corporativo – Titulo(s): _____
☐ Socio - ☐ Limitado ☐ General
☐ Abogado de hecho
☐ Fideicomisario
☐ Guardián o conservador
☐ Otro: _____

El firmante representa: _____

Huella índice derecho del firmante
Parte alta del dedo aquí

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Bogotá, D.C., Colombia
Norby Fernando Mora

AUTORIZACION

Genentech, Inc. por la presente autoriza a cualquiera de Janet E. Hasak, Mark T. Kresnak, Jeffrey S. Kubinec y Thomity R. Schwartz, mientras permanezcan como empleados de Genentech, Inc., a ejecutar, a su nombre, cualquier solicitud asociada con presentación, procesamiento, consecución, y extensión y mantenimiento de concesión de solicitudes de patente y marca registrada en los Estados Unidos y países extranjeros, contratos relacionados con depósitos biológicos con la Colección Americana de Tipo de Cultivo, y cesiones a publicistas de derechos de autor que cubran los trabajos de autoría de los empleados de Genentech.

27 de Agosto de 2002
Fechado

(Firma)
Sean A. Johnston
Vicepresidente, Propiedad Intelectual
Genentech, Inc.



Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America

This public document:

2. has been signed by Harriet Smith Windsor

3. acting in the capacity of Secretary of State of Delaware

4. bears the seal/stamp of Office of Secretary of State

Certified

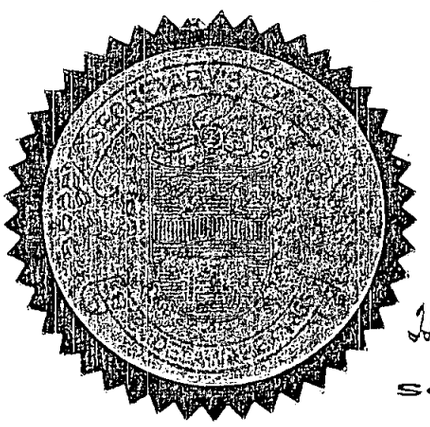
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254030

9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State

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NOTARIO CUARENTA (E)

Bogotá, D.C., Colombia

Norby Fernando Mora

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE RESTATED CERTIFICATE OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE TWENTY-SECOND DAY OF JULY, A.D. 1999, AT 9 O'CLOCK A.M.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

AUTHENTICATION: 3900384

050426506

DATE: 05-24-05

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NOTARIO CUARENTA

Bogotá, D.C., Colombia

Norby Fernando Mora S.

AMENDED AND RESTATED

CERTIFICATE OF INCORPORATION

GENENTECH, INC.

Genentech, Inc. (the "**Corporation**"), a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware, does hereby amend the Certificate of Incorporation of the Corporation, which was originally filed on December 23, 1986 under the name "Genentech Delaware, Inc.", and subsequently amended.

The Certificate of Incorporation of the Corporation is hereby restated and further amended to read in its entirety as follows:

ARTICLE 1

The name of the Corporation is: Genentech, Inc.

ARTICLE 2

The address of the registered office of the Corporation in the State of Delaware is Corporation Service Company, 1013 Centre Road, City of Wilmington, County of New Castle, Delaware 19805. The name of its registered agent at such address is Corporation Service Company.

ARTICLE 3

The purpose of the Corporation is to engage in any lawful act or activity for which corporations may be organized under the General Corporation Law of the State of Delaware as the same exists or may hereafter be amended ("**Delaware Law**").

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 09:00 AM 07/22/1999
991302331 – 2112549

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ARTICLE 4

SECTION 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is four hundred million (400,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("**Preferred Stock**"). Three hundred million (300,000,000) shares shall be designated common stock, par value $0.02 per share ("**Common Stock**") The Common Stock of the Corporation shall be all of one class.

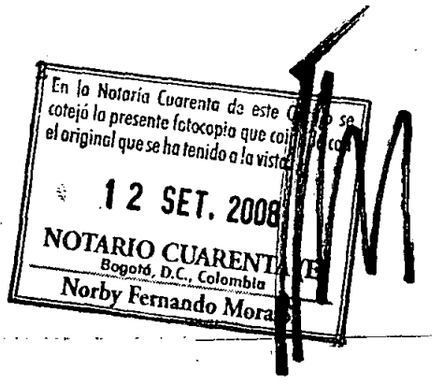
(b) The number of authorized shares of any class or classes of stock may be increased or decreased (but not below the number of shares thereof then outstanding) by the affirmative vote of a majority in voting power of the outstanding stock of the Corporation entitled to vote thereon irrespective of the provisions of Section 242(b)(2) of Delaware Law.

SECTION 4.02. *Common Stock.* (a) *Issuance and Consideration.* Any unissued or treasury shares of the Common Stock may be issued for such consideration as may be fixed in accordance with applicable law from time to time by the Board of Directors.

(b) *Dividends.* Subject to the rights of holders of the Preferred Stock, the holders of the Common Stock shall be entitled to receive, when and as declared by the Board of Directors, out of the assets of the Corporation which are by law available therefor, dividends payable either in cash, in property, or in shares of stock and the holders of the Preferred Stock shall not be entitled to participate in any such dividends (unless otherwise provided by the Board of Directors in any resolution providing for the issue of a series of Preferred Stock).

SECTION 4.03. *Preferred Stock.*

(a) *Series and Limits of Variations between Series.* Any unissued or treasury shares of the Preferred Stock may be issued from time to time in one or more series for such consideration as may be fixed from time to time by the Board of Directors. Before any shares of Preferred Stock of any particular series shall be issued, a certificate shall be filed with the Secretary of State of Delaware setting forth the designation, rights, privileges, restrictions, and conditions to be attached to the Preferred Stock of such series and such other matters as may be required, and the Board of Directors shall fix and determine, and is hereby expressly empowered to fix and determine, in the manner provided by law, the particulars of



the shares of such series (so far as not inconsistent with the provisions of this Article 4 applicable to all series of Preferred Stock), including, but not limited to, the following:

(i) the distinctive designation of such series and the number of shares which shall constitute such series, which number may be increased (except where otherwise provided by the Board of Directors in creating such series) or decreased (but not below the number of shares thereof then outstanding) from time to time by like action of the Board of Directors;

(ii) the annual rate of dividends payable on shares of such series, the conditions upon which such dividends shall be payable and the date from which dividends shall be cumulative in the event the Board of Directors determines that dividends shall be cumulative;

(iii) whether such series shall have voting rights, in addition to the voting rights provided by law and, if so, the terms of such voting rights;

(iv) whether such series shall have conversion privileges and, if so, the terms and conditions of such conversion, including, but not limited to, provision for adjustment of the conversion rate upon such events and in such manner as the Board of Directors shall determine;

(v) whether or not the shares of such series shall be redeemable and, if so, the terms and conditions of such redemption, including the date or dates upon or after which they shall be redeemable, and the amount per share payable in case of redemption, which amount may vary under different conditions and at different redemption dates;

(vi) whether such series shall have a sinking fund for the redemption or purchase of shares of that series and, if so, the terms and amount of such sinking fund;

(vii) the rights of the shares of such series in the event of voluntary or involuntary liquidation, dissolution or winding up of the Corporation, and the relative rights of priority, if any, of payment of shares of that series; and

(viii) any other relative rights, preferences and limitations of such series.

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ARTICLE 5

SECTION 5.01. *Amendment of Bylaws by Directors.* (a) In furtherance and not in limitation of the powers conferred by statute, the Board of Directors is expressly authorized to make, alter, amend, repeal and rescind the bylaws of the Corporation, subject to Sections 5.01(b) and 5.01(c) hereof.

(b) Sections 2.02, 3.02, 3.03(a), 3.03(b), 3.04, 3.05, 3.14, and 5.06 of the bylaws may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 60% of the shares entitled to be voted thereon.

(c) Sections 3.03(c) and 3.15 of the bylaws may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 90% of the shares entitled to be voted thereon.

SECTION 5.02. *Amendment of Certificate of Incorporation.* Section 5.01(b) of this Certificate of Incorporation may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 60% of the shares entitled to be voted thereon. Section 5.01(c) and Articles 8 and 9 of this Certificate of Incorporation may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 90% of the shares entitled to be voted thereon.

ARTICLE 6

SECTION 6.01. *Election of Directors by Holders of Preferred Stock.* During any period when the holders of any Preferred Stock or any one or more series thereof, voting as a class, shall be entitled to elect a specified number of directors, by reason of dividend arrearages or other provisions giving them the right to do so, then and during such time as such right continues (i) the then otherwise authorized number of directors shall be increased by such specified number of directors, and the holders of such Preferred Stock or such series thereof, voting as a class, shall be entitled to elect the additional directors so provided for, pursuant to the provisions of such Preferred Stock or series; (ii) each such additional director shall serve for such term, and have such voting powers, as shall be stated in the provisions pertaining to such Preferred Stock or series; and (iii) whenever the holders of any such Preferred Stock or series thereof are

4
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divested of such rights to elect a specified number of directors, voting as a class, pursuant to the provisions of such Preferred Stock or series, the terms of office of all directors elected by the holders of such Preferred Stock or series, voting as a class pursuant to such provisions or elected to fill any vacancies resulting from the death, resignation or removal of directors so elected by the holders of such Preferred Stock or series, shall forthwith terminate and the authorized number of directors shall be reduced accordingly.

SECTION 6.02. *Ballots*. Elections of directors at an annual or special meeting of stockholders need not be by written ballot unless the bylaws of the Corporation shall provide otherwise.

SECTION 6.03. *Elimination of Certain Personal Liability of Directors*. (a) A director of the Corporation shall not be liable to the Corporation or its stockholders for monetary damages for breach of fiduciary duty as a director to the fullest extent permitted by Delaware Law.

(b) Each person (and the heirs, executors or administrators of such person) who was or is a party or is threatened to be made a party to, or is involved in any threatened, pending or completed action, suit or proceeding, whether civil, criminal, administrative or investigative, by reason of the fact that such person is or was a director of the Corporation or is or was serving at the request of the Corporation as a director of another corporation, partnership, joint venture, trust or other enterprise, shall be indemnified and held harmless by the Corporation to the fullest extent permitted by Delaware Law. The right to indemnification conferred in this Section 6.03 shall also include the right to be paid by the Corporation the expenses incurred in connection with any such proceeding in advance of its final disposition to the fullest extent authorized by Delaware Law. The right to indemnification conferred in this Section 6.03 shall be a contract right.

(c) The Corporation may, by action of its Board of Directors, provide indemnification to such of the officers, employees and agents of the Corporation to such extent and to such effect as the Board of Directors shall determine to be appropriate and authorized by Delaware Law.

(d) The Corporation shall have power to purchase and maintain insurance on behalf of any person who is or was a director, officer, employee or agent of the Corporation, or is or was serving at the request of the Corporation as a director, officer, employee or agent of another corporation, partnership, joint venture, trust or other enterprise against any expense, liability or loss incurred by such person in any such capacity or arising out of his status as such, whether or

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not the Corporation would have the power to indemnify him against such liability under Delaware Law.

(e) The rights and authority conferred in this Section 6.03 shall not be exclusive of any other right which any person may otherwise have or hereafter acquire.

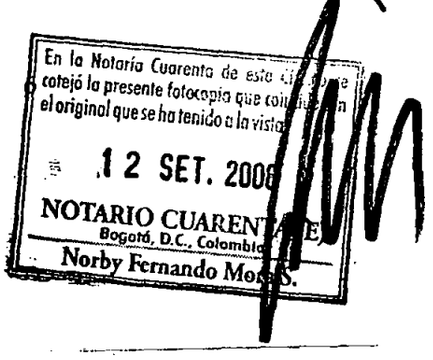
(f) Neither the alteration, amendment, repeal or recission of this Section 6.03, nor the adoption of any provision of this Certificate of Incorporation or the bylaws of the Corporation, nor, to the fullest extent permitted by Delaware Law, any modification of law, shall eliminate or reduce the effect of this Section 6.03 in respect of any acts or omissions occurring prior to such alteration, amendment, repeal, recission, adoption or modification.

ARTICLE 7

SECTION 7.01. *Section 203 of Delaware Law.* The Corporation hereby elects not to be governed by Section 203 of Delaware Law.

ARTICLE 8

SECTION 8.01. *Relationship With Roche.* In anticipation that the Corporation will cease to be a wholly owned subsidiary of Roche Holdings, Inc., but that Roche (as defined in Section 8.05) will remain a stockholder of the Corporation, and in anticipation that the Corporation (as defined in Section 8.05) and Roche may engage in the same or similar activities or lines of business and have an interest in the same areas of corporate opportunities, and in recognition of (i) the benefits to be derived by the Corporation through its continued contractual, corporate and business relations with Roche (including service of officers and directors of Roche as directors of the Corporation) and (ii) the difficulties attendant to any director, who desires and endeavors fully to satisfy such director's fiduciary duties, in determining the full scope of such duties in any particular situation, the provisions of this Article 8 are set forth to regulate, define and guide the conduct of certain affairs of the Corporation as they may involve Roche and its officers and directors, and the powers, rights, duties and liabilities



of the Corporation and its officers, directors and stockholders in connection therewith.

SECTION 8.02. *Similar Business Activities*. Except as Roche Holdings, Inc. may otherwise agree in writing,

(a) Roche shall not have a duty to refrain from engaging directly or indirectly in the same or similar business activities or lines of business as the Corporation, and

(b) neither Roche nor any officer or director thereof shall be liable to the Corporation or its stockholders for breach of any fiduciary duty by reason of any such activities of Roche or of such person's participation therein.

In the event that Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for both Roche and the Corporation, Roche shall have no duty to communicate or offer such corporate opportunity to the Corporation and shall not be liable to the Corporation or its stockholders for breach of any fiduciary duty as a stockholder of the Corporation or controlling person of a stockholder by reason of the fact that Roche pursues or acquires such corporate opportunity for itself, directs such corporate opportunity to another person or entity, or does not communicate information regarding, or offer, such corporate opportunity to the Corporation.

SECTION 8.03. *Corporate Opportunities*. In the event that a director, officer or employee of the Corporation who is also a director, officer or employee of Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for the Corporation and Roche (whether such potential transaction or matter is proposed by a third-party or is conceived of by such director, officer or employee of the Corporation), such director, officer or employee shall be entitled to offer such corporate opportunity to the Corporation or Roche as such director, officer or employee deems appropriate under the circumstances in his sole discretion, and no such director, officer or employee shall be liable to the Corporation or its stockholders for breach of any fiduciary duty or duty of loyalty or failure to act in (or not opposed to) the best interests of the Corporation or the derivation of any improper personal benefit by reason of the fact that (i) such director, officer or employee offered such corporate opportunity to Roche (rather than the Corporation) or did not communicate information regarding such corporate opportunity to the Corporation or (ii) Roche pursues or acquires such corporate opportunity for itself or directs such corporate opportunity to another person or does not communicate information regarding such corporate opportunity to the Corporation.

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Norby Fernando Mo[illegible] S.

SECTION 8.04. *Deemed Consent of Stockholders.* Any person or entity purchasing or otherwise acquiring any interest in any shares of capital stock of the Corporation shall be deemed to have notice of and to have consented to the provisions of this Article 8.

SECTION 8.05. *Definitions.* For purposes of this Article 8 and Article 9 only, (i) the term "**Corporation**" shall mean the Corporation and all corporations, partnerships, joint ventures, associations and other entities in which the Corporation beneficially owns (directly or indirectly) fifty percent or more of the outstanding voting stock, voting power or similar voting interests, and (ii) the term "**Roche**" shall mean Roche Holdings, Inc. and all corporations, partnerships, joint ventures, associations and other entities (other than the Corporation, defined in accordance with clause (i) of this Section 8.05) that are "**affiliates**" (as defined in Rule 12b-2 under the Securities Exchange Act of 1934, as amended) of Roche Holdings, Inc.

SECTION 8.06. *Termination; Binding Effect.* Notwithstanding anything in this Certificate of Incorporation to the contrary, the foregoing provisions of this Article 8 shall expire on the date that Roche ceases to own beneficially Common Stock representing at least 5% of the number of outstanding shares of Common Stock of the Corporation and no person who is a director or officer of the Corporation is also a director or officer of Roche. Neither such expiration, nor the alteration, amendment, change or repeal of any provision of this Article 8 nor the adoption of any provision of this Amended and Restated Certificate of Incorporation inconsistent with any provision of this Article 8 shall eliminate or reduce the effect of this Article 8 in respect of any matter occurring, or any cause of action, suit or claim that, but for this Article 8, would accrue or arise, prior to such expiration, alteration, amendment, repeal or adoption.

SECTION 8.07. *Article 9.* The provisions of this Article 8 are in addition to the provisions of Article 9.

ARTICLE 9

SECTION 9.01. *Contracts Not Void.* No contract, agreement, arrangement or transaction (or any amendment, modification or termination thereof) between the Corporation and Roche shall be void or voidable solely for the reason that Roche is a party thereto, or solely because any directors or officers of the Corporation who are affiliated with Roche are present at or participate in th

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Bogotá, D.C., Colombia
Norby Fernando Mora

meeting of the Board of Directors or committee thereof which authorizes the contract, agreement, arrangement, transaction, amendment, modification or termination or solely because his or their votes are counted for such purpose, but any such contract, agreement, arrangement or transaction (or any amendment, modification or termination thereof) shall be governed by the provisions of this Amended and Restated Certificate of Incorporation, the Corporation's Bylaws, Delaware Law and other applicable law. For purposes of this Article 9, the terms the "**Corporation**" and "**Roche**" have the meanings set forth in Section 8.05.

SECTION 9.02. *Quorum.* Directors of the Corporation who are also directors or officers of Roche may be counted in determining the presence of a quorum at a meeting of the Board of Directors or of a committee that authorizes or approves any such contract, agreement, arrangement or transaction (or amendment, modification or termination thereof). Outstanding shares of Common Stock owned by Roche may be counted in determining the presence of a quorum at a meeting of stockholders that authorizes or approves any such contract, agreement, arrangement or transaction (or amendment, modification or termination thereof).

SECTION 9.03. *No Liability For Good Faith Actions.* Neither Roche nor any officer or director thereof shall be liable to the Corporation or its stockholders for breach of any fiduciary duty or duty of loyalty or failure to act in (or not opposed to) the best interests of the Corporation or the derivation of any improper personal benefit by reason of the fact that Roche or an officer of director thereof in good faith takes any action or exercises any rights or gives or withholds any consent in connection with any agreement or contract between Roche and the Corporation. No vote cast or other action taken by any person who is an officer, director or other representative of Roche, which vote is cast or action is taken by such person in his capacity as a director of this Corporation, shall constitute an action of or the exercise of a right by or a consent of Roche for the purpose of any such agreement or contract.

SECTION 9.04. *Deemed Consent by Stockholders.* Any person or entity purchasing or otherwise acquiring any interest in any shares of capital stock of the Corporation shall be deemed to have notice of and to have consented to the provisions of this Article 9.

SECTION 9.05. *Contracts Covered.* For purposes of this Article 9, any contract, agreement, arrangement or transaction with the Corporation or any corporation, partnership, joint venture, association or other entity in which the Corporation beneficially owns (directly or indirectly) fifty percent or more of the outstanding voting stock, voting power or similar voting interests shall be deemed to be a contract, agreement, arrangement or transaction with the Corporation.

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Norby Fernando Mesa S.

SECTION 9.06. *Binding Effect.* Neither the alteration, amendment, change or repeal of any provision of this Article 9 nor the adoption of any provision inconsistent with any provision of this Article 9 shall eliminate or reduce the effect of this Article 9 in respect of any matter occurring, or any cause of action, suit or claim that, but for this Article 9, would accrue or arise, prior to such alteration, amendment, change, repeal or adoption.

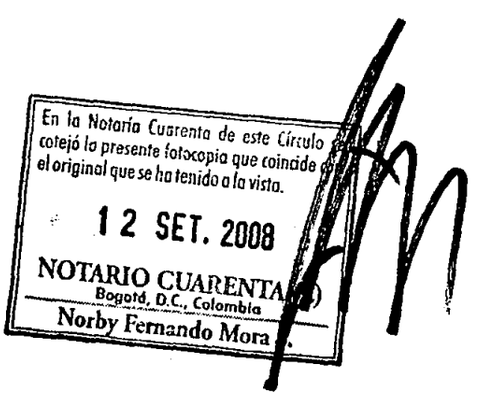
SECTION 9.07. *Article 8.* The provisions of this Article 9 are in addition to the provisions of Article 8.

En la Notaría Cuarenta de este [illegible] cotejó la presente fotocopia que corresponde al original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

IN WITNESS WHEREOF, this Amended and Restated Certificate of Incorporation, having been duly adopted in accordance with Sections 242 and 245 of the General Corporation Law of the State of Delaware and by the written consent of the sole stockholder of the Corporation in accordance with Section 228 of the General Corporation Law of the State of Delaware, has been executed this 22nd day of July, 1999.

GENENTECH, INC.

By: /s/ Stephen G. Juelsgaard
Name: Stephen G. Juelsgaard
Title: Senior Vice President,
General Counsel and Secretary



766

TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc.

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 Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254030
9. Sello/timbre
10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado



767

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO VUELTO A REDACTAR DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL VIGÉSIMO SEGUNDO DÍA DE JULIO, A. D. 1999, A LAS 9 EN PUNTO DE LA MAÑANA.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900384

FECHA: 05 – 24 – 05

CORREGIDO Y VUELTO A REDACTAR
CERTIFICADO DE INCORPORACIÓN
GENENTECH, INC.

Genentech, Inc (la "**Corporación**"), una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware, por la presente corrige el Certificado de Incorporación, el fue presentado originalmente el 23 de diciembre de 1986, bajo el nombre "Genentech Delaware, Inc.", y corregido subsiguientemente.

Por la presente se vuelve a redactar el certificado de incorporación de la Corporación y se corrige aun más para que diga en su totalidad lo que sigue:

ARTICULO 1

El nombre de la Corporación es: Genentech, Inc.

ARTICULO 2

La dirección de la oficina registrada de la Corporación en el Estado de Delaware es Corporation Service Company, 1013 Centre Road, Ciudad de Wilmington, Condado de New castle, Delaware 19805. El nombre de su agente registrado en tal dirección es Corporation Service Company.

ARTICULO 3

El propósito de a Corporación es el de participar en cualquier acto o actividad legal para las cuales las corporaciones pueden ser organizadas bajo la Ley General de Corporación del Estado de Delaware como existe o puede se modificada la misma en adelante ("**Delaware Law**").

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12 SET [illegible]
NOTARIO CUARENTA (E)
Bogotá, D.C. Colombia
Norby Fernando Mora S.

ARTICULO 4

SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de cuatrocientos millones (400,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones

preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Trecientos millones (300,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

(b) El número de acciones autorizadas de cualquier clase o clases de acciones puede incrementarse o disminuirse (pero no por debajo del número del número de acciones de las mismas que en su momento están vigentes) por el voto afirmativo de una mayoría con poder de votación de las acciones vigentes de la Corporación con derecho a votar por la misma independiente de las consideraciones de la Sección 242(b)(2) de la Ley de Delaware.

SECCION 4.02. *Acción Común.* (a) *Emisión y Consideración.* Cualquier acción sin emitir o del tesoro del las Acciones Comunes puede emitirse para tal consideración como pueda fijarse de acuerdo con la ley aplicable cada cierto tiempo por la Junta Directiva.

(b) *Dividendos.* Sujeto a los derechos de los accionistas de las Acciones Preferenciales, los accionistas de Acciones Comunes deberán tener el derecho a recibir, cuando y como se declare por la Junta Directiva, de los activos de la Corporación los cuales están disponibles para ello por ley, dividendos pagables ya sea en dinero, propiedad, o acciones y los accionistas de la Acción Preferencial no tendrán derecho a participar en tales dividendos (a menos que la Junta Directiva determine lo contrario en cualquier resolución que condiciona la emisión de una serie de Acciones Preferenciales).

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12 SET. 2008
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Bogotá, D.C. Colombia
Nora Y. Fernando Mora S.

SECCIÓN 4.03. *Acción Preferencial*

(a) *Series y Límites de Variaciones entre Series.* Cualquier acción no emitida o del tesoro de las Acciones Preferenciales puede emitirse cada cierto tiempo en una o más series para tal consideración como pueda ser fijada cada cierto tiempo por la Junta Directiva. Antes de que se emita cualquier acción de las Acciones Preferenciales de cualquier serie particular, se deberá presentar un certificado ante el Secretario de Estado de Delaware estableciendo las designaciones, derechos, privilegios, restricciones, y condiciones que irán anexas a las Acciones Preferenciales de tal serie y tales otras cuestiones como puedan requerirse, y la Junta Directiva deberá fijar y determinar, y por la presente está expresamente autorizada para fijar y determinar, en la forma provista por la ley, los

particulares de las acciones de tal serie (siempre y cuando no sean inconsistentes con las condiciones de este Artículo 4 aplicables a todas las series de Acciones Preferenciales), incluyendo, pero sin limitarse a, las siguientes:

(i) la designación distintiva de tal serie y el número de las acciones que han de constituir tal serie, numero que puede incrementarse (excepto cuando lo determine de otro modo la Junta Directiva al crear tal serie) o reducida (pero no por debajo del número de acciones de las mismas que están vigentes) cada cierto tiempo por una acción semejante de la Junta Directiva;

(ii) la tasa anual de dividendos a pagar sobre las acciones de tal serie, las condiciones sobre las cuales se pagarán tales dividendos y le fecha a partir de la cual los dividendos serán acumulativos en el evento que la Junta Directiva determine que los dividendos serán acumulativos;

(iii) si la serie tendrá o no derechos de voto, además de los derechos de voto provistos por la ley, y de ser así, los términos de tales derechos de voto;

(iv) si tal serie tendrá o no privilegios de conversión y, de ser así, los términos y condiciones de tal conversión, incluyendo, pero sin estar limitado a, la condición para ajuste de la tasa de conversión en tales eventos y de tal forma como la Junta Directiva lo determine;

(v) si las acciones de tal serie serán o no redimibles y, de ser así, los términos y condiciones de tal redención, incluyendo la fecha o fechas en las cuales o después de las cuales pueden ser redimibles, y la cantidad a pagar por acción en caso de redención, cantidad que variará bajo diferentes condiciones y en diferentes fechas de redención;

(vi) si tal serie tendrá o no un fondo de inversión para la redención o compra de acciones de esa serie y, de ser así, los términos y la cantidad de tal fondo de inversión;

(vii) los derechos de las acciones en caso de una liquidación, disolución o cierre voluntaria o involuntaria de la Corporación, y

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12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Eduardo Mo

los derechos relativos de prioridad, de haber, de pago de acciones de esa serie; y

(viii) cualquier otro derecho relativo, preferencia y limitación de tal serie.

ARTICULO 5

SECCION 5.01. *Enmiendas a los estatutos por los Directores.* (a) En adición y no limitando los poderes conferidos por estatutos, la Junta Directiva esta expresamente autorizada para hacer, alterar, enmendar, rechazar y rescindir los estatutos de la Corporación, sujetos a las secciones 5.01(b) y 5.01(c) a continuación.

(b) Las secciones 2.02, 3.02, 3.03(a), 3.04, 3.05, 3.14 y 5.06 de los estatutos solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 60% de las acciones con derecho a votar las mismas.

(c) Las secciones 3.03(c) y 3.15 de los estatutos solo pueden ser alteradas, enmendadas, repelidas, o rescindidas por le voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 90% de las acciones con derecho a votar las mismas.

SECCION 5.02. *Enmienda del Certificado de Incorporación.* La sección 5.01 (b) de este Certificado de Incorporación solo puede ser alterada, enmendada, repelida, o rescindida por el voto afirmativo de los accionistas de capital con derecho a voto sobre la misma que representen a más del 60% de las acciones con derecho a votar la misma. La sección 5.01(c) y los Artículos 8 y 9 de este Certificado de Incorporación solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar la misma que representen más del 90% de las acciones con derecho a votar la misma.

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12 SET. 2008
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Bogotá, D.C., Colombia
Norby Fernando Montes

ARTICULO 6

SECCION 6.01. *Elección del Directorio por los Accionistas de Acción Preferencial.* Durante cualquier periodo cuando los accionistas de cualquier

permita la Ley de Delaware. El derecho a la indemnización concedido en esta sección 6.03 también debe incluir el derecho a que la Corporación le pague los gastos incurridos en conexión cuna cualquiera de tales procesos por adelantado de su decisión final hasta donde los autoriza la Ley de Delaware. El derecho a indemnización conferido en esta sección 6.03 será un derecho contractual.

(c) La Corporación puede, por acción de su Junta Directiva, proporcionar indemnización a tales funcionarios, empleados y agentes de la Corporación hasta tal punto y a tal efecto como lo determine apropiado la Junta Directiva y lo autorice la Ley de Delaware.

(d) La Corporación tendrá el poder de comprar y mantener un seguro a nombre de cualquier persona quien es o fue director, funcionario, empleado o agente de la corporación, o esta o estuvo en servicio por pedido de la Corporación como director, funcionario, empleado o agente de otra corporación, sociedad, sociedad de riesgo compartido, fideicomiso u otra empresa contra cualquier gasto, responsabilidad o pérdida incurrida por tal persona en cualquier capacidad o que surge de su estado como tal, ya sea si la Corporación tiene o no el poder de indemnizarlo contra tal responsabilidad bajo la ley de Delaware.

(e) Los derechos y autoridad conferidos en esta Sección 6.03 no serán exclusivos de ningún otro derecho el cual cualquier otra persona pueda tener o adquirir de aquí en adelante.

(f) Ni la alteración, enmienda, rechazo o rescisión de esta Sección 6.03, ni la adopción de ninguna condición de este Certificado de Incorporación o de los Estatutos de la Corporación, ni, hasta donde lo permite la Ley de Delaware, ninguna modificación de la ley, debe eliminar o reducir el efecto de esta Sección 6.03 respecto a ninguno de los actos u omisiones que ocurran antes de tal alteración, enmienda, rechazo, rescisión, adopción o modificación.

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12 SET. 2008
NOTARIO CUARENTA
Bogotá, D.C., Colombia
Norby Fernando Mora S.

ARTICULO 7

SECCION 7.01, *Sección 203 de la Ley de Delaware.* Por la presente la Corporación elige no ser gobernada por la Sección 203 de la Ley de Delaware.

ARTICULO 8

SECCION 8.01. *Relación con Roche.* Anticipándose a que la Corporación cese de ser un subsidiaria completamente de propiedad de Roche Holdings, Inc., pero que Roche (como se define en la sección 8.05) permanezca siendo un accionista de la Corporación, y anticipándose a que la Corporación (como se define en la Sección 8.05) y Roche puede entrar en actividades o líneas de negocios semejantes o similares y tener un interés en las mismas áreas de oportunidades corporativas, y en reconocimiento de (i) los beneficios a ser derivados por la Corporación a través de sus relaciones contractuales, corporativas y de negocios con Roche (incluyendo el servicio de funcionarios y directores de Roche como directores de la Corporación) y (ii) las dificultades concomitantes a cualquier director, quien desea y se propone satisfacer completamente tales deberes fiduciarios del director, al determinar el alcance completo de tales deberes en cualquier situación particular, las condiciones de este artículos 8 se establecen para regular, definir y guiar la conducta de ciertas cuestiones de la Corporación ya que pueden involucrar a Roche y sus funcionaros y directores, y los poderes, derechos, deberes y responsabilidades de la Corporación y sus funcionarios, directores y accionistas en conexión con éste.

SECCION 8.02. *Actividades en Negocios Similares.* Excepto que Roche Holdings, Inc. pueda de otra forma acordarlo por escrito,

(a) Roche no tendrá el deber de abstenerse de entrar directamente o indirectamente en actividades de negocios o líneas de negocios semejantes o similares a las de la Corporación, y

(b) ni Roche ni ninguno de sus funcionarios o director de la misma será responsable a la Corporación o sus accionistas por violar cualquier deber fiduciario por razón de alguna de tales actividades de Roche o de la participación de tal persona en las mismas.

En el evento que Roche adquiera conocimientos de una transacción o materia potencial que pueda ser una oportunidad corporativa tanto para Roche como para la Corporación, Roche no tendrá el deber de comunicar u ofrecer tal oportunidad corporativa a la Corporación y no será responsable ante la Corporación o sus accionistas por violar ningún deber fiduciario como accionista de la Corporación o la persona que controla a un accionista por razón del hacho

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NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Libby Fernando Mora S.

que Roche prosigue o adquiere tal oportunidad corporativa para si misma, dirige tal oportunidad corporativa a otra persona o entidad, o no comunica información respecto, u ofrece, tal oportunidad corporativa a la Corporación.

SECCION 8.03. *Oportunidades Corporativas.* En el evento que un director, funcionario o empleado de la Corporación quien también es director, funcionario o empleado de Roche adquiera conocimiento de una transacción o materia potencial que pueda ser una oportunidad corporativa para la Corporación y Roche (ya se si esta transacción o materia potencial es propuesta por un tercero o es concebida por tal director, funcionario o empleado de la Corporación o no), tal director, funcionario o empleado debe tener el derecho a ofrecer tal oportunidad corporativa a la Corporación o Roche como tal director, funcionario o empleado los considere apropiado bajo las circunstancias de su discreción, y tal director, funcionario o empleado no será responsable a la Corporación o sus accionistas por violar ningún deber fiduciario o deber de lealtad o faltar en actuar en (o no oponerse a) los mejores intereses de la Corporación o la derivación de ningún beneficio personal inapropiado por razón del hecho que (i) tal director, funcionario o empleado ofreció tal oportunidad corporativa a Roche (en lugar de a la Corporación) o no comunicó información respecto a tal oportunidad corporativa a la Corporación o (ii) Roche busca o adquiere tal oportunidad corporativa para si misma o dirige tal oportunidad corporativa a otra persona o no comunica información respecto a tal oportunidad corporativa a la Corporación.

SECCION 8.04. *Consentimiento estimado de los accionistas.* Se considerará que cualquier persona o entidad que compre o de otro modo adquiera algún interés en cualquiera de las acciones de capital de la Corporación ha tenido noticia o ha estado de acuerdo con las condiciones de este Artículo 8.

NOTARIO CUARENTA (E) Bogotá, D.C. Colombia
12 SET. 2008

SECCION 8.05, *Definiciones.* Para propósitos de este Artículo 8 y el Artículo 9 solamente, (i) el término ***"Corporación"*** debe significar la Corporación y todas las corporaciones, sociedades, sociedades de riesgo compartido y otras entidades en las cuales la Corporación posee con beneficio (directa o indirectamente) cincuenta por ciento o más de las acciones con voto, poder de voto o intereses de voto similares, y (ii) el término ***"Roche"*** debe significar Roche Holdings, Inc y todas las corporaciones, sociedades, sociedades de riesgo compartido, asociaciones y otras entidades (diferentes a la Corporación, definida de acuerdo con la cláusula (i) de esta sección 8.05) que son ***"afiliados"*** (como se

define en la Regla 12b-2 bajo el Acta de Intercambio de Valores de 1934, como se enmendó) de Roche Holdings, Inc.

SECCION 8.06, *Finalización: Efecto Obligante.* Son consideración con nada en este Certificado de Incorporación que lo contradiga, las condiciones anteriores de este Artículo 8 deberán expirar en la fecha que Roche cese de poseer con beneficios Acciones Comunes que representen al menos 5% del número de acciones vigentes de las Acciones Comunes de la Corporación y nadie que se director o funcionario de la Corporación sea también director o funcionario de Roche. Ni tal vencimiento, ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 8 así como tampoco ninguna condición de este Certificado de Incorporación Enmendado y Vuelto a Redactar inconsistente con alguna de las condiciones de este Artículo 8 eliminará o reducirá el efecto de este Artículo 8 respecto a cualquier materia, o cualquier causa de acción, demanda o reclamo que, excepto por este Artículo 8, pueda acaecer o surgir, antes de tal vencimiento, alteración, enmienda o adopción.

SECCION 8.07. *Artículo 9.* Las condiciones de este Artículo son adicionales a las condiciones del Artículo 9.

ARTICULO 9

SECCION 9.01. *Contratos no nulos.* Ningún contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización de los mismos) entre la Corporación y Roche será nulo u anulable solamente por razón de que Roche es parte del mismo, o solamente porque cualquiera de los directores o funcionarios de la Corporación quienes están afiliados a Roche están presentes en o participan en la reunión de la Junta Directiva o comité de la misma que autoriza el contrato, acuerdo, arreglo, transacción, enmienda, modificación o finalización o solamente porque su o sus votos se cuentan para tal propósito, pero cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo) estará gobernado por las condiciones de este Cerificado de Incorporación Enmendado y vuelto a Redactar, los Estatutos de la Corporación, la Ley de Delaware y otras leyes aplicables. Para propósitos de este Artículo 9, los términos "***Corporación***" y "***Roche***" tienen los significados establecidos en la Sección 8.05.

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cotejó la presente fotocopia que coincide con
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Bogotá, D.C., Colombia
Norby Fernando Mora S.

SECCION 9.02. *Quórum.* Los Directores de la Corporación que son también directores o funcionarios de Roche pueden contarse al determinar la presencia de un quórum en una reunión de la Junta Directiva o de un comité que autoriza o aprueba cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo). Las acciones vigentes de las Acciones Comunes de propiedad de Roche pueden contarse al determinar la presencia de un quórum en una reunión de accionistas que autorice o apruebe tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo).

SECCION 9.03. *No responsabilidad por acciones de buena fe.* Ni Roche ni ningún funcionario o director de la misma será responsable ante la Corporación o sus accionistas por violar cualquiera de los deberes fiduciarios o deberes de lealtad o por fallar en actuar en (o no oponerse a) los mejores intereses de la Corporación o por derivación de cualquier beneficio personal por razón del hecho que Roche o un funcionario o director de la misma de buena fe tome cualquier acción o ejerza cualquier derecho o de o retenga cualquier consentimiento en conexión con cualquier acuerdo o contrato entre Roche y la Corporación. Ningún voto dado u otra acción tomada por cualquier persona que es un funcionario, director o representante de Roche, donde el voto o acción es tomado por tal persona en su capacidad como director de esta Corporación, constituirá una acción de o el ejercicio de un derecho o un consentimiento de Roche para los propósitos de cualquiera de tal acuerdo o contrato.

SECCION 9.04. *Consentimiento estimado de los accionistas.* Se considerará que cualquier persona o entidad que compre o de otro modo adquiera algún interés en cualquiera de las acciones de capital de la Corporación ha tenido noticia o ha estado de acuerdo con las condiciones de este Artículo 9.

SECCION 9.05. *Contratos cubiertos.* Para propósitos de este Artículo 9, cualquier contrato, acuerdo, arreglo o transacción con la Corporación o cualquier corporación, sociedad, sociedad de riesgo conjunto, asociación u otra entidad en la cual la Corporación posee con beneficio (directa o indirectamente) el cincuenta por ciento o más de las acciones con voto, poderes de voto o intereses de voto similares vigentes se considerará un contrato, acuerdo o transacción con la Corporación.

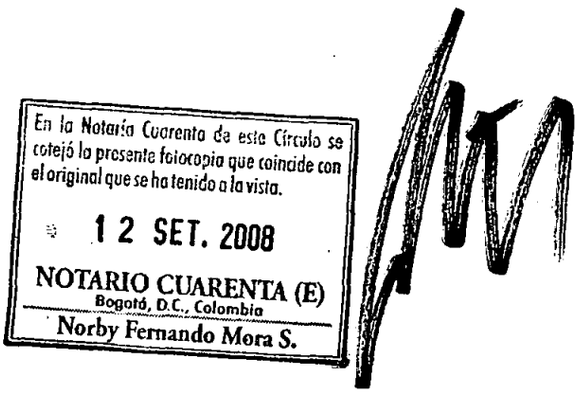
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12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

SECCION 9.06. *Efecto obligante.* Ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 9 ni la adopción de alguna condición inconsistente con cualquier condición de este Artículo 9 eliminará o reducirá el efecto de este Artículo 9 respecto a cualquier materia que ocurra, o cualquier causa de acción, demanda o reclamo que, a no ser por este Artículo 9, pueda acaecer o surgir, antes de tal alteración, enmienda, cambio, rechazo o adopción.

SECCION 9.07. *Artículo 8.* Las condiciones de este Artículo 9 son adicionales a las condiciones del Artículo 8.

EN TESTIMONIO DE LO CUAL, este Certificado de Incorporación Enmendado y vuelto a Redactar, el cual ha sido adoptado de acuerdo con las secciones 242 y 245 de la Ley General de Corporación del Estado de Delaware y por consentimiento escrito de el único accionista de la Corporación de acuerdo con la sección 228 de Ley General de Corporación del Estado de Delaware, ha sido ejecutado este 22 de Julio de 1999.

GENENTECH, INC.
Por: /s/ Stephen G. Juelsgaard
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente
Consejo General y Secretaría



97.200
779

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. *Country: United States of America*

This public document:

2. *has been signed by Harriet Smith Windsor*

3. *acting in the capacity of Secretary of State of Delaware*

4. *bears the seal/stamp of Office of Secretary of State*

Certified

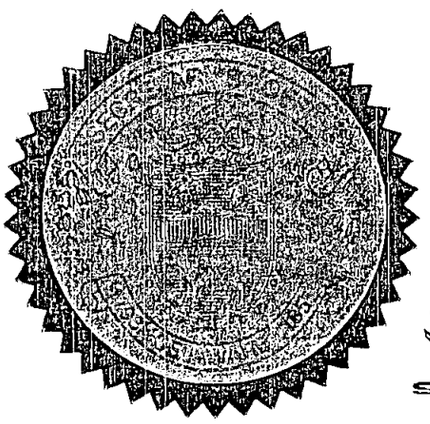
5. *at Dover, Delaware*

6. *the twenty-fourth day of May, A.D. 2005*

7. *by Secretary of State, Delaware Department of State*

8. *No. 0254031*

9. *Seal/Stamp:*



10. *Signature:*

Harriet Smith Windsor

Secretary of State

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

780

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE FIFTEENTH DAY OF MAY, A.D. 2000, AT 5 O'CLOCK P.M.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

AUTHENTICATION: 3900385

050426506

DATE: 05-24-05

En la Notaría Cuarenta de este [illegible] cotejó la presente fotocopia que corresponde con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora[illegible]

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 05:00 PM 05/15/2000
001245251 – 2112549

781

CERTIFICATE OF AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> "Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is seven hundred million (700,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Six hundred million (600,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 15th day of May, 2000.

GENENTECH, INC.

By: Stephen Juelsgaard

Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel and Secretary

75841

En la Notaría Cuarenta de este Circu
cotejó la presente fotocopia que coincide
el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (
Bogotá, D.C., Colombia
Norby Fernando Mora S.

782

TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc.

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 Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254031
9. Sello/timbre
10. Firma

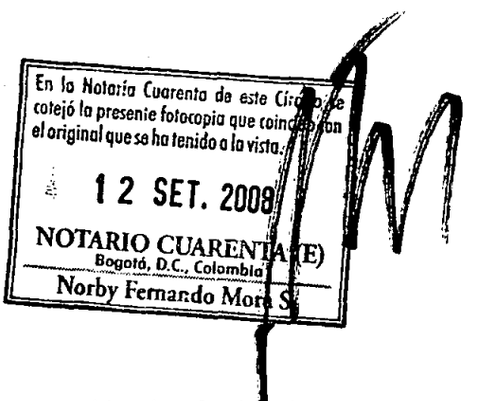
Harriet Smith Windsor (Firma)
Secretaria de Estado

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO VUELTO A REDACTAR DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMOQUINTO DIA DE MAYO, A. D. 2000, A LAS 5 EN PUNTO DE LA TARDE.



Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900385

FECHA: 05 – 24 – 05

CERTIFICADO DE ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de setecientos millones (700,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Seiscientos millones (600,000,000) de acciones serán designadas acciones comunes, por un valor de $0.01 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

En la Notaría Cuarenta y Uno de Bogotá D.C. cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA Y UNO
Bogotá, D.C. Colombia
Norby Fernando Marcó

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de su Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente y se sostuvo al ser anunciada de acuerdo con la sección 222 de la Ley General de Corporación del Estado de Delaware, en donde el número necesario de acciones requerido por los estatutos votaron a favor de la enmienda.

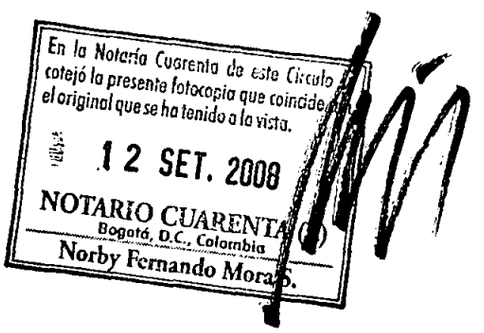
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 15 de Mayo de 2000.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.



786

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America

This public document:

2. has been signed by Harriet Smith Windsor

3. acting in the capacity of Secretary of State of Delaware

4. bears the seal/stamp of Office of Secretary of State

Certified

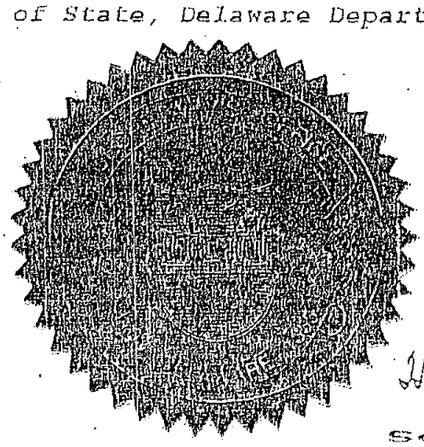
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254033

9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que corresponde con el original que se ha tenido a la vista.

12 SET. 2005

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando [illegible] S.

787

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE SIXTEENTH DAY OF APRIL, A.D. 2004, AT 7:39 O'CLOCK P.M.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA
Bogotá, D.C., Colombia
Norby Fernando Mora S.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900387

DATE: 05-24-05

788

State of Delaware
Secretary of State
Division of Corporations
Delivered 07:44 PM 04/16/2004
FILED 07:39 PM 04/16/2004
SRV 040281669 - 2112549 FILE

CERTIFICATE OF THIRD AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc., a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware,

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is three billion one hundred million (3,100,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Three billion (3,000,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The common stock of the Corporation shall be all of one class.

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Third Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 16th day of April, 2004.

GENENTECH, INC.

By: _____

Name: Stephen G. Juelsgaard

Title: Executive Vice President, General Counsel and Secretary

#153087

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que corresponde con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mar[illegible] S.

789

TRÁDUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

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 Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado.

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254033
9. Sello/timbre
10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

790

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO DE ENMIENDA DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMOSEXTO DIA DE ABRIL, A. D. 2004, A LAS 7:39 EN PUNTO DE LA TARDE.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado

CERTIFICADO DE TERCERA ENMIENDA DEL CERTIFICADO DE INCORPORACIÓN CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de tres mil cien millones (3,100,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Tres mil millones (3,000,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Norby Fernando Mo[illegible] S.

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de la Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente

TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 16 de Abril de 2004.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

793

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America

This public document:

2. has been signed by Harriet Smith Windsor

3. acting in the capacity of Secretary of State of Delaware

4. bears the seal/stamp of Office of Secretary of State

Certified

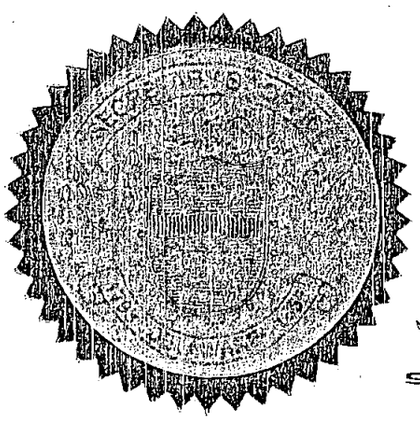
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254032

9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2006

NOTARIO CUARENTA (E)

Bogotá, D.C., Colombia

Norby Fernando Mora S.

794

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE TENTH DAY OF MAY, A.D. 2001, AT 2:05 O'CLOCK P.M.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900386

DATE: 05-24-05

TARY OF STATE
V OF CORPORATIONS
2:05 PM 05/10/2001
20224 - 2112549

S ////STATE/OF/DELAWARE///// V
T ///SECRETARY/OF/STATE///// O
A DIVISION/OF/CORPORATIONS// I
M FILED/09:00/AM/05/10/2001/ D
P ///010226224//F/2112549////
BY Sandy Miller

795

CERTIFICATE OF AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> "Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is one billion three hundred million (1,300,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). One billion two hundred million (1,200,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 10th day of May, 2001.

GENENTECH, INC.

By: Stephen Juelsgaard

Name: Stephen G. Juelsgaard

Title: Senior Vice President, General Counsel and Secretary

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

TRADUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

APOSTILLA

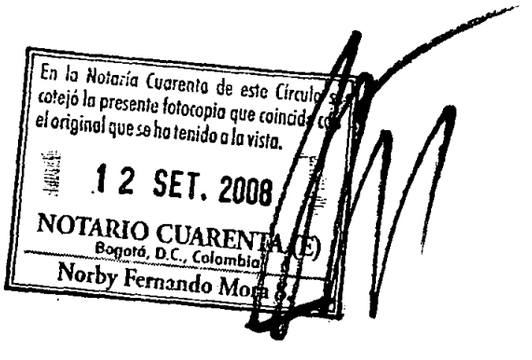
(Convención de La Haya del 5 de Octubre de 1961)

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254032
9. Sello/timbre
10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado



797

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO DE ENMIENDA DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMO DIA DE MAYO, A. D. 2001, A LAS 2:05 EN PUNTO DE LA TARDE.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 SET. 2008

NOTARIO CUARENTA (E)
Bogotá, D.C., Colombia
Norby Fernando Mora S.

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900386

FECHA: 05 – 24 – 05

798

CERTIFICADO DE TERCERA ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de mil trescientos millones (1,300,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Mil doscientos millones (1,200,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción con derecho a voto de la Corporación será toda de una misma clase.

En la Notaría Cuarenta de este Círculo se cotejó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 SET. 2008
NOTARIO CUARENTA (E)
Bogotá, D.C. Colombia
Norby Fernando Mejía G.

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de su Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente y se sostuvo al ser anunciada de acuerdo con la sección 222 de la Ley General de Corporación del Estado de Delaware, en donde el número necesario de acciones requerido por los estatutos votaron a favor de la enmienda.

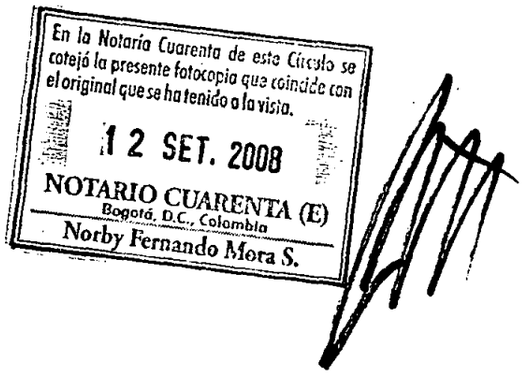
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 10 de Mayo de 2001.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.



REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

gotá,
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ctor(a)
DENA SARMIENTO JUAN PABLO
oderado(a) y/o Representante de
NENTECH, INC.

FERENCIA	Radicación No.	8 127238
	Solicitud internacional	PCT/US2007/069889
	Fecha inicio fase nacional	30/12/2008
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	0598

mo resultado del examen de forma, realizado con fundamento en el artículo 38 de Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de operación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular ica de la Superintendencia de Industria y Comercio, se le comunica que:

No se aceptan las modificaciones presentadas en el folio 615, porque no rresponden a modificaciones efectuadas en la fase internacional (Art 19 y 34 del T) estas podrán presentarse una vez haya entrado en fase nacional, cancelando la sa correspondiente. Adicionalmente se recuerda al solicitante que, en caso de alizar modificaciones, debe presentar un nuevo capítulo reivindicatorio completo e incluya las reivindicaciones que se modifican y las que no.

La solicitud no contiene la copia del documento en que consta la cesión del recho a la patente del inventor al solicitante o a su causante, conforme a lo tablecido por el artículo 26 literal k) de la Decisión 486 de la Comisión de la munidad Andina. Cuando se trate de documentos otorgados en el extranjero, estos berán legalizarse de conformidad con lo dispuesto por los artículos 259 y 260 del digo de Procedimiento Civil. Cuando se trate de las declaraciones a que hace ferencia la Regla 4.17 ii) del Tratado de Cooperación en Materia de Patentes T., el alcance de las tales declaraciones se determinará según indiquen aramente una cesión de derechos del inventor al solicitante o su causante, de nformidad con lo dispuesto en el numeral 5.2.15 del Capítulo Quinto, Título X de Circular Única de Superintendencia de Industria y Comercio.

cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le quiere para que dentro del término de dos meses siguientes a la fecha de tificación del presente oficio, complete los requisitos anteriormente enunciados. caso de no dar cumplimiento al presente requerimiento, la solicitud se nsiderará abandonada y perderá su prelación.

tifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal , del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

Continua en la página siguiente...

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Radicación No.:8 127238

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 23 ENE. 2009
jfernand