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DOMICILIO SOUTH SAN FRANCISCO, CALIFORNIA, ESTADOS UNIDOS

74. **APODERADO** JUAN PABLO CADENA SARMIENTO

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1 SOLICITUD DE:

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2 SOLICITANTE (71)	Nombre: **GENENTECH, INC.** Dirección: 1 DNA Way, South San Francisco, CA 94080-4990, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail	**IDENTIFICACION** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual_____ Número _____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Carrera 7 # 71 – 21 Torre B – Piso 4 Teléfono: 744-2200 Fax: 744-2700 E-mail:	**IDENTIFICACION** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual_____ Número 80.410.337 TP 57492
4 INVENTOR (ES) (72)	Nombre: Allen J. EBENS, JR., Frederic S. JACOBSON, Paul POLAKIS, Ralph H. SCHWALL, Mark X. SLIWKOWSKI, Susan D. SPENCER Dirección: 1932 Arroyo Ave, San Carlos, CA 94070 (US), 830 Shattuck Avenue, Berkeley, CA 94707 (US), 1449 Cortez Avenue, Burlingame, CA 94010 (US), 400 Griffin Avenue, Pacifica, CA 94044 (US), 42 Oak Creek Lane, San Carlos, CA 94070 (US), 2395 Paradise Drive, Tiburon, CA 94920 (US) (Respectivamente) Nacionalidad o Domicilio: Estados Unidos de América (Respectivamente)	

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10 ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud. $463.000
- [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.
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- [ ] 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.
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11 FIGURA CARACTERÍSTICA:

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(54) Title: ANTIBODY DRUG CONJUGATES AND METHODS

(57) Abstract: The present invention relates to antibody-drug conjugate compounds of Formula I: Ab-(L-D)p where one or more maytansinoid drug moieties (D) are covalently linked by L to an antibody (Ab) which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors. These compounds may be used in methods of diagnosis or treatment of cancer, and other diseases and disorders.

ANTIBODY-DRUG CONJUGATES AND METHODS

This non-provisional application filed under 37 CFR §1.53(b), claims the benefit under 35 USC §119(e) of US Provisional Application Ser. No. 60/576,517 filed on June 1, 2004 and US Provisional Application Ser. No. 60/616,098 filed on October 5, 2004, each of which are incorporated by reference in their entirety.

FIELD OF THE INVENTION

The invention relates generally to compounds with anti-cancer activity and more specifically to antibodies conjugated with chemotherapeutic maytansinoid drugs or toxins. The invention also relates to methods of using antibody-drug conjugate compounds for *in vitro*, *in situ*, and *in vivo* diagnosis or treatment of mammalian cells, or associated pathological conditions.

BACKGROUND OF THE INVENTION

Antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders. The use of antibody-drug conjugates (ADC), i.e. immunoconjugates, for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Payne, G. (2003) Cancer Cell 3:207-212; Trail et al (2003) Cancer Immunol. Immunother. 52:328-337; Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drug Del. Rev. 26:151-172; US 4975278) theoretically 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. (eds), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Efforts to design and refine ADC have focused on the selectivity of monoclonal antibodies (mAbs) as well as drug-linking and drug-releasing properties. 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, mitomycin, neocarzinostatin (Takahashi et al (1988) Cancer 61:881-888) 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 (US 4753894; US 5629197; US 4958009; US 4956453), small molecule toxins such as geldanamycin (Mandler et al (2000) J. 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.

An antibody-radioisotope conjugate has been approved, ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) composed of a murine IgG1 kappa monoclonal antibody directed against CD20 antigen and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. J. 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). 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 4970198; US 5079233; US 5585089; US 5606040; US 5693762; US 5739116; US 5767285; US 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 (Xie et al (2004) J. of Pharm. and Exp. Ther. 308(3):1073-1082; Tolcher et al (2003) J. Clin. Oncology 21(2):211-222; US 5208020), underwent Phase I trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.) is an antibody-drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, 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" (also, see: US 4981979). HERCEPTIN® (trastuzumab) linked by SMCC to DM1 has been reported (WO 2005/037992).

In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides, i.e. tumor-associated antigens (TAA), has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Monoclonal antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders. An example of successful antibody therapy is HERCEPTIN® (trastuzumab), a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd = 5 nM) to the extracellular domain of the human epidermal growth factor receptor2 protein, HER2 (ErbB2) (US 5821337; US 6054297; US 6407213; US 6639055; Coussens L, et al (1985) Science 230:1132-9; Slamon DJ, et al (1989) Science 244:707-12). Trastuzumab is an IgG1 kappa antibody that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. Trastuzumab binds to the HER2 antigen and thus inhibits the growth of cancerous cells. Because trastuzumab is a humanized antibody, it minimizes any HAMA response in patients. The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary, CHO) suspension culture. The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of

185kDa, which is structurally related to the epidermal growth factor receptor. HER2 protein overexpression is observed in 25%–30% of primary breast cancers and can be determined using an immunohistochemistry based assessment of fixed tumor blocks (Press MF, et al (1993) Cancer Res 53:4960-70. Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2 (Hudziak RM, et al (1989) Mol Cell Biol 9:1165-72; Lewis GD, et al (1993) Cancer Immunol Immunother; 37:255-63; Baselga J, et al (1998) Cancer Res. 58:2825-2831). Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity, ADCC (Hotaling TE, et al (1996) [abstract]. Proc. Annual Meeting Am Assoc Cancer Res; 37:471; Pegram MD, et al (1997) [abstract]. Proc Am Assoc Cancer Res; 38:602; Sliwkowski et al (1999) Seminars in Oncology 26(4), Suppl 12:60-70; Yarden Y. and Sliwkowski, M. (2001) Nature Reviews: Molecular Cell Biology, Macmillan Magazines, Ltd., Vol. 2:127-137). HERCEPTIN® is clinically active in patients with ErbB2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga et al, (1996) J. Clin. Oncol. 14:737-744). Although HERCEPTIN is a breakthrough in treating patients with ErbB2-overexpressing breast cancers that have received extensive prior anti-cancer therapy, the majority of the patients in this population fail to respond or respond only poorly to HERCEPTIN treatment. Therefore, there is a significant clinical need for developing further HER2-directed cancer therapies for those patients with HER2-overexpressing tumors or other diseases associated with HER2 expression that do not respond, or respond poorly, to HERCEPTIN treatment. In addition to HER2, there is an opportunity to exploit other tumor-associated antigens with targeted therapies.

SUMMARY

The present invention provides novel compounds with biological activity against cancer cells. The compounds may inhibit tumor growth in mammals and may be useful for treating human cancer patients.

The present invention relates to the delivery, transport, accumulation or retention of therapeutic antibody-drug conjugate (ADC) compounds inside cells. The invention is more particularly related to attaining high concentrations of active metabolite molecules in cancer cells. Intracellular targeting may be achieved by methods and compounds which allow accumulation or retention of biologically active agents inside cells. Such effective targeting may be applicable to a variety of therapeutic formulations and procedures.

The surprising discovery has been made that antibody-drug conjugates with stable, non-disulfide linkers groups that attach a maytansinoid drug moiety to an antibody result in increased *in vitro* potency and *in vivo* efficacy. In addition, the antibody-drug conjugates show the unexpected result of better safety *in vivo* relative to certain disulfide linker conjugates.

Antibody-drug conjugate (ADC) compounds comprise an antibody covalently attached by a linker to one or more maytansinoid drug moieties. ADC may be represented by Formula I:

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

where one or more maytansinoid drug moieties (D) are covalently linked by L to an antibody (Ab). Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors. The linker L may be stable outside a cell, i.e. extracellular. The linker L, the maytansinoid drug moiety D, or the linker and the maytansinoid drug moiety taken together (L-D), do not comprise a disulfide group.

In one embodiment, a substantial amount of the drug moiety is not cleaved from the antibody until the antibody-drug conjugate enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate, and the drug moiety is cleaved from the antibody when the antibody-drug conjugate does enter the cell.

In another embodiment, the ADC specifically binds to a receptor encoded by an ErbB gene, such as EGFR, HER2, HER3 and HER4. The ADC may specifically bind to the extracellular domain of the HER2 receptor. The ADC may inhibit growth of tumor cells which overexpress HER2 receptor.

In another embodiment, the antibody (Ab) of Formula I is a humanized antibody such as huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 or huMAb4D5-8 (trastuzumab).

Another aspect of the invention is a pharmaceutical composition including a Formula I compound, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable diluent, carrier, or excipient.

Another aspect provides a pharmaceutical combination comprising a Formula I compound and a second compound having anti-cancer properties or other therapeutic effects..

Another aspect includes diagnostic and therapeutic uses for the compounds and compositions disclosed herein.

Another aspect is a method for killing or inhibiting the proliferation of tumor cells or cancer cells comprising treating the cells with an amount of an antibody-drug conjugate, or a pharmaceutically acceptable salt or solvate thereof, being effective to kill or inhibit the proliferation of the tumor cells or cancer cells.

Another aspect are methods of treating cancer comprising administering to a patient a formulation of a Formula I compound. One method is for the treatment of cancer in a mammal, wherein the cancer is characterized by the overexpression of an ErbB receptor. The mammal optionally does not respond, or responds poorly, to treatment with an unconjugated anti-ErbB antibody. The method comprises administering to the mammal a therapeutically effective amount of an antibody-drug conjugate compound.

Another aspect is a method of inhibiting the growth of tumor cells that overexpress a growth factor receptor selected from the group consisting of HER2 receptor and EGF receptor comprising administering to a patient an antibody-drug conjugate compound which binds specifically to said growth factor receptor and a chemotherapeutic agent wherein said antibody-drug conjugate and said chemotherapeutic agent are each administered in amounts effective to inhibit growth of tumor cells in the patient.

Another aspect is a method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor, comprising administering a combination of an antibody-drug conjugate compound of Formula I and a chemotherapeutic agent.

Another aspect is an assay method for detecting cancer cells comprising: exposing cells to an antibody-drug conjugate compound, and determining the extent of binding of the antibody-drug conjugate compound to the cells.

Another aspect concerns methods of screening ADC drug candidates for the treatment of a disease or disorder where the disease or disorder is characterized by the overexpression of HER2.

Another aspect includes articles of manufacture, i.e. kits, comprising an antibody-drug conjugate, a container, and a package insert or label indicating a treatment.

Another aspect includes methods of treating a disease or disorder characterized by the overexpression of HER2 in a patient with the antibody-drug conjugate compounds.

Another aspect includes methods of making, methods of preparing, methods of synthesis, methods of conjugation, and methods of purification of the antibody-drug conjugate compounds, and the intermediates for the preparation, synthesis, and conjugation of the antibody-drug conjugate compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an *in vitro*, cell proliferation assay with SK-BR-3 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 2 shows an *in vitro*, cell proliferation assay with BT-474 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 3 shows an *in vitro*, cell proliferation assay with MCF7 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 4 shows an *in vitro*, cell proliferation assay with MDA-MB-468 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 5 shows the serum clearance in beige nude mice without tumors of trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, measuring conjugate and total antibody serum concentration at six time points (5 minutes, 1 hour, 6 hours, 24 hours, 72, 168 hours post-dose) over 7 days.

Figure 6 shows the stability over time in nude mice without tumors of the conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, and trastuzumab-SMCC-DM1, measuring serum concentration at six time points (5 minutes, 1 hour, 6 hours, 24 hours, 72, 168 hours post-dose) over 7 days.

Figure 7 shows the measurement of serum concentrations of total trastuzumab / trastuzumab-SMCC-DM1, and total trastuzumab / trastuzumab-SPP-DM1 in mice, 7 days after treatment, with and without tumor.

Figure 8 shows a plasma concentration clearance study after administration of 10 mg/kg of trastuzumab-SPP-DM1 to 4 subject rats. Concentrations of total antibody and trastuzumab-SPP-DM1 were measured. (tr = trastuzumab)

Figure 9 shows a plasma concentration clearance study after administration of 10 mg/kg of trastuzumab-SMCC-DM1 to 4 subject rats. Concentrations of total antibody and trastuzumab-SMCC-DM1 were measured.

Figure 10 shows the mean tumor volume change over time in mice dosed with: Vehicle (PBS pH 6.5), trastuzumab-SPP-DM1 (370 μg DM1/m^2), and trastuzumab-SMCC-DM1 (330 μg DM1/m^2) where dose refers to the dose of DM1 administered.

Figure 11 shows the mean tumor volume change over time in athymic nude mice with Fo5 tumor allografts dosed on Day 0 with: Vehicle (PBS pH 6.5), 10 mg/kg trastuzumab-SIAB-DM1 (3.4 DM1/Ab; 168 μg DM1/kg), and 10 mg/kg trastuzumab-SMCC-DM1 (3.2 DM1/Ab; 158 μg DM1/kg) where dose refers to the dose amount of the antibody-drug conjugate administered.

Figure 12 shows the mean tumor volume change over time in MMTV-Her2 Fo 5 beige nude mice (seven each group, all with tumors, Ti = 7) by single injection with Vehicle (PBS pH 6.5), 10 mg/kg trastuzumab-

SPP-DM1, 10 mg/kg trastuzumab-SPP-DM4, 10 mg/kg trastuzumab-SPP-DM3, and 10 mg/kg trastuzumab-SMCC-DM1.

Figure 13 shows the time to double tumor volume and log cell kill analysis for Vehicle (PBS pH 6.5), trastuzumab-SPP-DM1, trastuzumab-SPP-DM4, trastuzumab-SPP-DM3, and trastuzumab-SMCC-DM1 in HER2-Fo5 tumors.

Figure 14 shows the change in body weight over time of rats dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (3260 μg DM1/m^2), and free DM1 (650 μg/m^2).

Figure 15 shows a liver function test measured in AST units per liter over time in the rat model dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), and free DM1.

Figure 16 shows a safety profile measured in PLT units in cells per liter over time in the rat model dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), and free DM1.

Figure 17 shows an in vitro, cell proliferation assay with HT1080EphB2 (C8) cells treated with antibody-drug conjugates: –▲– antiEphB2R 2H9-SPP-DM1, and –▼– antiEphB2R 2H9-SMCC-DM1.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs, and are consistent with: Singleton *et al.*, (1994) *Dictionary of Microbiology and Molecular Biology, 2nd Ed.*, J. Wiley & Sons, New York, NY; and Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity (Miller et al (2003) Jour. of Immunology 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, *i.e.*, a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (*e.g.*, IgG, IgE, IgM, IgD, and IgA), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597; for example.

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 (US 4816567; and Morrison et al (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey or Ape) and human constant region sequences.

An "intact antibody" herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more "effector functions" which 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. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

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

An "ErbB receptor" is a receptor protein tyrosine kinase which belongs to the ErbB receptor family which are important mediators of cell growth, differentiation and survival. The ErbB receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or $p185^{neu}$), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). A panel of anti-ErbB2 antibodies has been characterized using the human breast tumor cell line SKBR3 (Hudziak et al (1989) Mol. Cell. Biol. 9(3):1165-1172. Maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α (US 5677171). The anti-ErbB2 antibodies discussed in Hudziak et al are further characterized in Fendly et al (1990) Cancer Research 50:1550-1558; Kotts et al. (1990) In Vitro 26(3):59A; Sarup et al. (1991) Growth Regulation 1:72-82; Shepard et al. J. (1991) Clin. Immunol. 11(3):117-127; Kumar et al. (1991) Mol. Cell. Biol. 11(2):979-986; Lewis et al. (1993) Cancer Immunol. Immunother. 37:255-263; Pietras et al. (1994) Oncogene 9:1829-1838; Vitetta et al. (1994) Cancer Research 54:5301-5309; Sliwkowski et al. (1994) J. Biol. Chem. 269(20):14661-14665; Scott et al. (1991) J. Biol. Chem. 266:14300-5; D'souza et al. Proc. Natl. Acad. Sci. (1994) 91:7202-7206; Lewis et al. (1996) Cancer Research 56:1457-1465; and Schaefer et al. (1997) Oncogene 15:1385-1394.

Other anti-ErbB2 antibodies with various properties have been described in Franklin et al (2004) Cancer Cell 5:317-328; Tagliabue et al (1991) Int. J. Cancer 47:933-937; McKenzie et al (1989) Oncogene 4:543-548; Maier et al (1991) Cancer Res. 51:5361-5369; Bacus et al (1990) Molecular Carcinogenesis 3:350-362; Stancovski et al (1991) PNAS (USA) 88:8691-8695; Bacus et al (1992) Cancer Research 52:2580-2589; Xu et al (1993) Int. J. Cancer 53:401-408; WO94/00136; Kasprzyk et al (1992) Cancer Research 52:2771-2776;

Hancock et al (1991) Cancer Res. 51:4575-4580; Shawver et al (1994) Cancer Res. 54:1367-1373; Arteaga et al (1994) Cancer Res. 54:3758-3765; Harwerth et al (1992) J. Biol. Chem. 267:15160-15167; US 5783186; and Klapper et al (1997) Oncogene 14:2099-2109.

Sequence identity screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (US 5,183,884; US 5,480,968; Kraus et al (1989) PNAS (USA) 86:9193-9197) and ErbB4 (EP 599274; Plowman et al (1993) Proc. Natl. Acad. Sci. USA, 90:1746-1750; and Plowman et al (1993) Nature 366:473-475). Both of these receptors display increased expression on at least some breast cancer cell lines.

The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a "native sequence" ErbB receptor or an "amino acid sequence variant" thereof. The ErbB receptor may be native sequence human ErbB receptor. Accordingly, a "member of the ErbB receptor family" is EGFR (ErbB1), ErbB2, ErbB3, ErbB4 or any other ErbB receptor currently known or to be identified in the future.

The terms "ErbB1", "epidermal growth factor receptor", "EGFR" and "HER1" are used interchangeably herein and refer to EGFR as disclosed, for example, in Carpenter et al (1987) Ann. Rev. Biochem. 56:881-914, including naturally occurring mutant forms thereof (e.g., a deletion mutant EGFR as in Humphrey et al., (1990) PNAS (USA), 87:4207-4211). The term erbB1 refers to the gene encoding the EGFR protein product. Antibodies against HER1 are described, for example, in Murthy et al (1987) Arch. Biochem. Biophys., 252:549-560 and in WO 95/25167.

The term "ERRP", "EGF-Receptor Related Protein", "EGFR Related Protein" and "epidermal growth factor receptor related protein" are used interchangeably herein and refer to ERRP as disclosed, for example in US 6399743 and US 2003/0096373.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al (1985) PNAS (USA), 82:6497-6501 and Yamamoto et al (1986) Nature, 319:230-234 (Genbank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat p185neu.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in US 5183884; US 5480968; Kraus et al (1989) PNAS (USA) 86:9193-9197. Antibodies against ErbB3 are known in the art (US 5183884; US 5480968; WO 97/35885).

The terms "ErbB4" and "HER4" herein refer to the receptor polypeptide as disclosed, for example, in EP Pat Appln No 599,274; Plowman et al., Proc. Natl. Acad. Sci. USA, 90:1746-1750 (1993); and Plowman et al., Nature, 366:473-475 (1993), including isoforms thereof, *e.g.*, as disclosed in WO 99/19488. Antibodies against HER4 are described, for example, in WO 02/18444.

Antibodies to ErbB receptors are available commercially from a number of sources, including, for example, Santa Cruz Biotechnology, Inc., California, USA.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand may be a native sequence human ErbB ligand such as epidermal growth factor (EGF) (Savage et al (1972) J. Biol. Chem., 247:7612-7621); transforming growth factor alpha (TGF-α) (Marquardt et al (1984) Science 223:1079-1082); amphiregulin also known as schwanoma or keratinocyte autocrine growth factor (Shoyab et al (1989) Science 243:1074-1076; Kimura et al (1990) Nature 348:257-260; and Cook et al (1991)

Mol. Cell. Biol., 11:2547-2557); betacellulin (Shing et al (1993) Science 259:1604-1607; and Sasada et al (1993) Biochem. Biophys. Res. Commun. 190:1173); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama et al (1991) Science 251:936-939); epiregulin (Toyoda et al (1995) J. Biol. Chem. 270:7495-7500; and Komurasaki et al (1997) Oncogene 15:2841-2848); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway et al., Nature, 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang et al (1997) Proc. Natl. Acad. Sci., 94:9562-9567); neuregulin-4 (NRG-4) (Harari et al (1999) Oncogene, 18:2681-89) or cripto (CR-1) (Kannan et al (1997) J. Biol. Chem., 272(6):3330-3335). ErbB ligands which bind EGFR include EGF; TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins. The ErbB ligand may also be a synthetic ErbB ligand. The synthetic ligand may be specific for a particular ErbB receptor, or may recognize particular ErbB receptor complexes. An example of a synthetic ligand is the synthetic heregulin/EGF chimera biregulin (see, for example, Jones et al (1999) FEBS Letters, 447:227-231, which is incorporated by reference).

"Heregulin" (HRG) refers to a polypeptide encoded by the heregulin gene product as disclosed in US 5641869 or Marchionni et al (1993) Nature 362:312-318. Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes et al (1992) Science 256:1205-1210; and US 5641869); neu differentiation factor (NDF) (Peles et al (1992) Cell 69: 205-216); acetylcholine receptor-inducing activity (ARIA) (Falls et al (1993) Cell 72:801-815); glial growth factors (GGFs) (Marchionni et al (1993) Nature, 362:312-318); sensory and motor neuron derived factor (SMDF) (Ho et al (1995) J. Biol. Chem. 270:14523-14532); γ-heregulin (Schaefer et al (1997) Oncogene, 15:1385-1394). The term includes biologically active fragments and/or amino acid sequence variants of a native sequence HRG polypeptide, such as an EGF-like domain fragment thereof (*e.g.*, HRGβ177-244).

"ErbB hetero-oligomer" is a noncovalently associated oligomer comprising at least two different ErbB receptors. An "ErbB dimer" is a noncovalently associated oligomer that comprises two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand. ErbB oligomers, such as ErbB dimers, can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al (1994) J. Biol. Chem., 269(20):14661-14665, for example. Examples of such ErbB hetero-oligomers include EGFR-ErbB2 (also referred to as HER1/HER2), ErbB2-ErbB3 (HER2/HER3) and ErbB3-ErbB4 (HER3/HER4) complexes. Moreover, the ErbB hetero-oligomer may comprise two or more ErbB2 receptors combined with a different ErbB receptor, such as ErbB3, ErbB4 or EGFR (ErbB1). Other proteins, such as a cytokine receptor subunit (*e.g.*, gp130) may be included in the hetero-oligomer.

By "ligand activation of an ErbB receptor" is meant signal transduction (e.g., that caused by an intracellular kinase domain of an ErbB receptor phosphorylating tyrosine residues in the ErbB receptor or a substrate polypeptide) mediated by ErbB ligand binding to a ErbB hetero-oligomer comprising the ErbB receptor of interest. Generally, this will involve binding of an ErbB ligand to an ErbB hetero-oligomer which activates a kinase domain of one or more of the ErbB receptors in the hetero-oligomer and thereby results in phosphorylation of tyrosine residues in one or more of the ErbB receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s). ErbB receptor activation can be quantified using various tyrosine phosphorylation assays.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (*e.g.*, ErbB receptor or ErbB ligand) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants will possess at least about 70% sequence identity with at least one receptor binding domain of a native ErbB ligand or with at least one ligand binding domain of a native ErbB receptor, or at least about 80%, or at least about 90% homologous with such receptor or ligand binding domains. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

"Sequence identity" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2," authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, (1991) Annu. Rev. Immunol, 9:457-92. To assess ADCC activity of a molecule of interest, an in vitro ADCC assay may be performed (US 5500362l; US 5821337). 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 (1998) PNAS (USA), 95:652-656.

"Maytansinoid drug moiety" means the substructure of an antibody-drug conjugate that has the structure of a maytansine compound. Maytansine was first isolated from the east African shrub *Maytenus serrata* (US 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (US 4151042). Synthetic maytansinol and maytansinol analogues have been reported. See US Patent Nos. 4137230; 4248870; 4256746; 4260608; 4265814; 4294757; 4307016; 4308268; 4308269; 4309428; 4313946; 4315929; 4317821; 4322348; 4331598; 4361650; 4364866; 4424219; 4450254; 4362663; and 4371533, and Kawai et al (1984) Chem. Pharm. Bull. 3441-3451), each of which are expressly incorporated by reference.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody, such as a native sequence human FcR. FcR may bind an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these 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 review M. in 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 (1994) Immunomethods, 4:25-34; and de Haas et al (1995) J. Lab. Clin. Med., 126:330-41. Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al (1976) J. Immunol. 117:587, and Kim et al (1994) J. Immunol., 24:249).

"Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (*e.g.*, an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al (1996) J. Immunol. Methods, 202:163, may be performed.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

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

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al supra*) and/or those residues from a "hypervariable loop" (*e.g.*, residues 26-32 (L1), 50-52

(L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk (1987) J. Mol. Biol., 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

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

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies 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.

"Single-chain Fv" or "scFv" mean single chain variable region antibody fragments which comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. The Fv polypeptide may further comprise a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding (Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). Anti-ErbB2 antibody scFv fragments are described in WO 93/16185; US 5571894; US 5587458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (VH) connected to a variable light 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 are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448.

"Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues.

Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al (1986) Nature, 321:522-525; Riechmann et al (1988) Nature 332:323-329; and Presta, (1992) Curr. Op. Struct. Biol., 2:593-596.

Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®, trastuzumab) as described in Table 3 of US 5821337 expressly incorporated herein by reference; humanized 520C9 (WO 93/21319) and humanized 2C4 antibodies.

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 diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. The antibody may be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, or more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup protein sequencer, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using 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 antibody "which binds" an antigen of interest, *e.g.*, ErbB2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. Where the antibody is one which binds ErbB2, it will usually preferentially bind ErbB2 as opposed to other ErbB receptors, and may be one which does not significantly cross-react with other proteins such as EGFR, ErbB3 or ErbB4. In such embodiments, the extent of binding of the antibody to these non-ErbB2 proteins (*e.g.*, cell surface binding to endogenous receptor) will be less than 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). Sometimes, the anti-ErbB2 antibody will not significantly cross-react with the rat neu protein, *e.g.*, as described in Schecter et al (1984) Nature 312:513, and Drebin et al (1984) Nature, 312:545-548.

An antibody which "blocks" ligand activation of an ErbB receptor reduces or prevents such activation, wherein the antibody is able to block ligand activation of the ErbB receptor substantially more effectively than monoclonal antibody 4D5, *e.g.*, about as effectively as monoclonal antibodies 7F3 or 2C4 or Fab fragments thereof. For example, the antibody that blocks ligand activation of an ErbB receptor may be one which is about 50-100% more effective than 4D5 at blocking formation of an ErbB hetero-oligomer. Blocking of ligand activation of an ErbB receptor can occur by any means, *e.g.*, by interfering with: ligand binding to an ErbB receptor, ErbB complex formation, tyrosine kinase activity of an ErbB receptor in an ErbB complex and/or phosphorylation of tyrosine kinase residue(s) in or by an ErbB receptor.

An antibody having a "biological characteristic" of a designated antibody, such as the monoclonal antibody designated 2C4 (Omnitarg, Genentech, Inc.), is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen (*e.g.*, ErbB2). For example, an antibody with a biological characteristic of 2C4 may block HRG activation of an ErbB hetero-oligomer comprising ErbB2 and ErbB3, ErbB1 or ErbB4; block EGF, TGF-α, HB-EGF, epiregulin and/or amphiregulin activation of an ErbB receptor comprising EGFR and ErbB2; block EGF, TGF-α and/or HRG mediated activation of MAPK; and/or bind the same epitope in the extracellular domain of ErbB2 as that bound by 2C4 (*e.g.*, which blocks binding of monoclonal antibody 2C4 to ErbB2).

Unless indicated otherwise, the expression "monoclonal antibody 2C4" refers to an antibody that has antigen binding residues of, or derived from, the murine 2C4 antibody of the Examples below. For example, the monoclonal antibody 2C4 may be murine monoclonal antibody 2C4 or a variant thereof, such as humanized antibody 2C4, possessing antigen binding amino acid residues of murine monoclonal antibody 2C4 (WO 01/00245). Unless indicated otherwise, the expression "rhuMAb 2C4" when used herein refers to an antibody comprising the variable light (VL) and variable heavy (VH) sequences of SEQ ID Nos. 3 and 4, respectively, fused to human light and heavy IgG1 (non-A allotype) constant region sequences optionally expressed by a Chinese Hamster Ovary (CHO) cell (WO 01/00245).

Unless indicated otherwise, the term "monoclonal antibody 4D5" refers to an antibody that has antigen binding residues of, or derived from, the murine 4D5 antibody (ATCC CRL 10463). For example, the monoclonal antibody 4D5 may be murine monoclonal antibody 4D5 or a variant thereof, such as a humanized 4D5, possessing antigen binding residues of murine monoclonal antibody 4D5. Exemplary humanized 4D5 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as in US 5821337.

A "growth inhibitory agent" refers to a compound or composition which inhibits growth of a cell, e.g. an ErbB expressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of ErbB expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest (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). Examples of "growth inhibitory" antibodies are those which bind to ErbB2 and inhibit the growth of cancer cells overexpressing ErbB2. Growth inhibitory anti-ErbB2 antibodies may inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, or greater than 50% (e.g., from about 50% to about 100%) at an antibody concentration of about 0.5 to 30 μg/ml, where the growth inhibition is determined six days after exposure of the SK-BR-3 cells to the antibody (US 5677171).

An antibody which "induces cell death" is one which causes a viable cell to become nonviable. The cell is generally one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. The cell may be a cancer cell, *e.g.*, a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death *in vitro* may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using

heat inactivated serum (*i.e.*, in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al (1995) Cytotechnology, 17:1-11) or 7AAD can be assessed relative to untreated cells. Cell death-inducing antibodies are those which induce PI uptake in the PI uptake assay in BT474 cells (see below).

An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses the ErbB2 receptor, including a tumor cell, *e.g.*, a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell.

The terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "disorder" is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemia and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders. An exemplary disorder to be treated in accordance with the present invention is a solid, malignant tumor

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may: (i) reduce the number of cancer cells; (ii) reduce the tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; and/or (vi) relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. In animal models, efficacy may be assessed by physical measurements of the tumor during the course following administration of the ADC, and by determining partial and complete remission of tumor. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

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

An "ErbB-expressing cancer" is one comprising cells which have ErbB protein present at their cell surface. An "ErbB2-expressing cancer" is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer.

A cancer which "overexpresses" a receptor, e.g. an ErbB receptor, is one which has significantly higher levels of the receptor, such as ErbB2, at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB protein present on the surface of a cell (*e.g.*, via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB-encoding nucleic acid in the cell, *e.g.*, via fluorescent in situ hybridization (FISH; see WO 98/45479), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Overexpression of the ErbB ligand, may be determined diagnostically by evaluating levels of the ligand (or nucleic acid encoding it) in the patient, *e.g.*, in a tumor biopsy or by various diagnostic assays such as the IHC, FISH, southern blotting, PCR or in vivo assays described above. One may also study ErbB receptor overexpression by measuring shed antigen (*e.g.*, ErbB extracellular domain) in a biological fluid such as serum (see, *e.g.*, US 4933294; WO 91/05264; US 5401638; and Sias et al (1990) J. Immunol. Methods 132: 73-80). Aside from the above assays, various other in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.*, a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.*, by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The tumors overexpressing HER2 are rated by immunohistochemical scores corresponding to the number of copies of HER2 molecules expressed per cell, and can be determined biochemically: 0 = 0-10,000 copies/cell, 1+ = at least about 200,000 copies/cell, 2+ = at least about 500,000 copies/cell, 3+ = about 1-2 x 10^6 copies/cell. Overexpression of HER2 at the 3+ level, which leads to ligand-independent activation of the tyrosine kinase (Hudziak et al., (1987) Proc. Natl. Acad. Sci. USA 84:7159-7163), occurs in approximately 30% of breast cancers, and in these patients, relapse-free survival and overall survival are diminished (Slamon et al

(1989) Science 244:707-712; Slamon et al (1987) Science, 235:177-182). Conversely, a cancer which is "not characterized by overexpression of the ErbB2 receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB2 receptor compared to a noncancerous cell of the same tissue type. The murine monoclonal anti-HER2 antibody inhibits the growth of breast cancer cell lines that overexpress HER2 at the 2+ and 3+ (1-2 x 10^6 HER2 receptors per cell) level, but has no activity on cells that express lower levels of HER2 (Lewis et al (1993) Cancer Immunol. Immunother. 37:255-263). Based on this observation, antibody 4D5 was humanized (huMAb4D5-8, rhuMAb HER2, US 5821337; Carter et al (1992) Proc. Natl. Acad. Sci. USA 89: 4285-4289) and tested in breast cancer patients whose tumors overexpress HER2 but who had progressed after conventional chemotherapy (Cobleigh et al (1999) J. Clin. Oncol. 17: 2639-2648). Most patient tumors in this trial expressed HER2 at the 3+ level, though a fraction was 2+.

A "hormone independent" cancer is one in which proliferation thereof is not dependent on the presence of a hormone which binds to a receptor expressed by cells in the cancer. Such cancers do not undergo clinical regression upon administration of pharmacological or surgical strategies that reduce the hormone concentration in or near the tumor. Examples of hormone independent cancers include androgen independent prostate cancer, estrogen independent breast cancer, endometrial cancer and ovarian cancer. Such cancers may begin as hormone dependent tumors and progress from a hormone-sensitive stage to a hormone-refractory tumor following anti-hormonal therapy.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.*, ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{60}C, and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogs and derivatives thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Erlotinib (TARCEVA®, Genentech/OSI Pharm.), Bortezomib (VELCADE®, Millenium Pharm.), Fulvestrant (FASLODEX®, Astrazeneca), Sutent (SU11248, Pfizer), Letrozole (FEMARA®, Novartis), Imatinib mesylate (GLEEVEC®, Novartis), PTK787/ZK 222584 (Novartis), Oxaliplatin (Eloxatin®, Sanofi), 5-FU (5-fluorouracil), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (GSK572016, GlaxoSmithKline), Lonafarnib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs.), and Gefitinib (IRESSA®, Astrazeneca), AG1478, AG1571 (SU 5271; Sugen), 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 trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); 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; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g.,

calicheamicin, especially calicheamicin gammaII and calicheamicin omegaI1 (Angew Chem Intl. Ed. Engl. (1994) 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, 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, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 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; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; 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® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON· toremifene; (ii) 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; (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) aromatase inhibitors; (v) protein kinase inhibitors; (vi) lipid kinase

inhibitors; (vii) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in abberant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (viii) ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME® ribozyme) and a HER2 expression inhibitor; (ix) vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; (x) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and (xi) pharmaceutically acceptable salts, acids or derivatives of any of the above.

Protein kinase inhibitors include tyrosine kinase inhibitors which inhibit to some extent tyrosine kinase activity of a tyrosine kinase such as an ErbB receptor. Examples of tyrosine kinase inhibitors include EGFR-targeted drugs such as: (i) antibodies which bind to EGFR, including MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US 4943533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBITUX®, Imclone) and reshaped human 225 (H225) (WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (US 5212290); humanized and chimeric antibodies that bind EGFR (US 5891996); and human antibodies that bind EGFR, such as ABX-EGF (WO 98/50433); (ii) anti-EGFR antibody conjugated with a cytotoxic agent (EP 659439A2); and small molecules that bind to EGFR including ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen), quinazolines such as PD 153035,4-(3-chloroanilino) quinazoline, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706, and pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-*d*] pyrimidines, curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide), tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (*e.g.*, those that bind to ErbB-encoding nucleic acid); quinoxalines (US 5804396); tyrphostins (US 5804396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-ErbB inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (Gleevac; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxanib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in: US 5804396; WO 99/09016 (American Cyanamid); WO 98/43960 (American Cyanamid); WO 97/38983 (Warner Lambert); WO 99/06378 (Warner Lambert); WO 99/06396 (Warner Lambert); WO 96/30347 (Pfizer, Inc); WO 96/33978 (Zeneca); WO 96/3397 (Zeneca); and WO 96/33980 (Zeneca).

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. An exemplary anti-angiogenic agent is an antibody that binds to Vascular Endothelial Growth Factor (VEGF) such as bevacizumab (AVASTIN®, Genentech).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor;

prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-ErbB2 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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

A "cardioprotectant" is a compound or composition which prevents or reduces myocardial dysfunction (*i.e.*, cardiomyopathy and/or congestive heart failure) associated with administration of a drug, such as an anthracycline antibiotic and/or an anti-ErbB2 antibody, to a patient. The cardioprotectant may, for example, block or reduce a free-radical-mediated cardiotoxic effect and/or prevent or reduce oxidative-stress injury. Examples of cardioprotectants encompassed by the present definition include the iron-chelating agent dexrazoxane (ICRF-187) (Seifert et al., *The Annals of Pharmacotherapy*, 28:1063-1072 (1994)); a lipid-lowering agent and/or anti-oxidant such as probucol (Singal et al., *J. Mol. Cell Cardiol.*, 27:1055-1063 (1995)); amifostine (aminothiol 2-[(3-aminopropyl)amino]ethanethiol-dihydrogen phosphate ester, also called WR-2721, and the dephosphorylated cellular uptake form thereof called WR-1065) and S-3-(3-methylaminopropylamino)propylphosphorothioic acid (WR-151327), see Green et al., (1994) Cancer Research,

54:738-741; digoxin (Bristow, M.R. ed. (1980) *Drug-Induced Heart Disease*. New York: Elsevier 191-215); beta-blockers such as metoprolol (Hjalmarson et al (1994) Drugs 47:Suppl 4:31-9; and Shaddy et al (1995) Am. Heart J., 129:197-9); vitamin E; ascorbic acid (vitamin C); free radical scavengers such as oleanolic acid, ursolic acid and N-acetylcysteine (NAC); spin trapping compounds such as alpha-phenyl-tert-butyl nitrone (PBN); (Paracchini et al (1993) Anticancer Res., 13:1607-1612); selenoorganic compounds such as P251 (Elbesen); and the like.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

"Alkyl" is C_1-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples of alkyl radicals include C_1-C_8 hydrocarbon moieties such as, but not limited to: methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$.

"Alkenyl" is C_2-C_{18} 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 of alkenyl radicals include C_2-C_8 hydrocarbon moieties such as, but not limited to: ethylene or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$), cyclopentenyl ($-C_5H_7$), and 5-hexenyl ($-CH_2CH_2CH_2CH_2CH=CH_2$)

"Alkynyl" is C_2-C_{18} 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 of alkynyl radicals include C_2-C_8 hydrocarbon moieties such as, but not limited to: acetylenic ($-C\equiv CH$) and propargyl ($-CH_2C\equiv CH$),

"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 C_1-C_8 hydrocarbon moieties such

as, 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.

"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 C_2-C_8 hydrocarbon moieties such as, 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 C_2-C_8 hydrocarbon moieties such as, but are not limited to: acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Aryl", alone or in combination, means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Aryl radical may contain one, two or three rings wherein such rings may be attached together in a pendent manner, e.g. biphenyl, or may be fused, e.g. napthalene or anthracene. Some aryl groups are represented in the exemplary structures as "Ar". Typical aryl groups include C_6-C_{12} hydrocarbon moieties such as, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"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.

Alkyl, alkylene, aryl, arylalkyl, and heteroarylalkyl groups may be substituted where one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O$^-$, -OR, -SR, -S$^-$, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, -NC(=O)R, -C(=O)R, -C(=O)NR_2, -SO_3^-, -SO_3H, -S(=O)$_2$R, -OS(=O)$_2$OR, -S(=O)$_2$NR, -S(=O)R, -OP(=O)(OR)$_2$, -P(=O)(OR)$_2$, -PO^-_3, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2^-, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR_2, -C(=S)NR_2, -C(=NR)NR_2, where each X is independently a halogen: F, Cl,

Br, or I; and each R is independently H, C_1–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", also known as heterocycle or heterocyclyl, refers to a ring system radical in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heteroaryl radical comprises 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heteroaryl 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. Heteroaryl compounds 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.

"Linker" 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. Linkers include a divalent radical such as an alkylene, an arylene, a heteroarylene, moieties such as: $-(CR_2)_nO(CR_2)_n-$, repeating units of alkyloxy (e.g. polyethyleneoxy, 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.

The phrase "pharmaceutically acceptable salt," as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an ADC. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

"Pharmaceutically acceptable solvate" refers to an association of one or more solvent molecules and an ADC. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

ANTIBODY-DRUG CONJUGATES

The compounds of the invention include those with utility for anticancer activity. In particular, the compounds include an antibody conjugated, i.e. covalently attached by a linker, to a drug moiety where the drug when not conjugated to an antibody has a cytotoxic or cytostatic effect. The biological activity of the drug moiety is thus modulated by conjugation to an antibody. The antibody-drug conjugates (ADC) of the invention may selectively deliver an effective dose of a cytotoxic agent to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose may be achieved.

In one embodiment, the bioavailability of the ADC, or an intracellular metabolite of the ADC, is improved in a mammal when compared to the corresponding maytansinoid compound alone. Also, the bioavailability of the ADC, or an intracellular metabolite of the ADC is improved in a mammal when compared to the corresponding antibody alone (antibody of the ADC, without the drug moiety or linker).

In one embodiment, the maytansinoid drug moiety of the ADC is not cleaved from the antibody until the antibody-drug conjugate binds to a cell-surface receptor or enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate. The drug moiety may be cleaved from the antibody after the antibody-drug conjugate enters the cell. The maytansinoid drug moiety may be intracellularly cleaved in a mammal from the antibody of the compound, or an intracellular metabolite of the compound, by enzymatic action, hydrolysis, oxidation, or other mechanism. For example, and in no way meant to limit the invention to a particular mechanism of action, the sulfur atom of the maytansinoid drug moiety of the ADC may be oxidized to a sulfone or sulfoxide group. Protons on carbons bound to the sulfone and sulfoxide may be removed under general or enzymatic catalysis inside the cell and result in a beta-elimination fragmentation that cleaves and separates the drug moiety from the antibody of the ADC. Alternatively, other electron withdrawing groups such as amides in the linker, antibody or drug moiety may effect similar fragmentation/cleavage mechanisms inside a cell.

Antibody-drug conjugates (ADC) may be represented by Formula I:

$$Ab–(L–D)_p \quad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GHDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

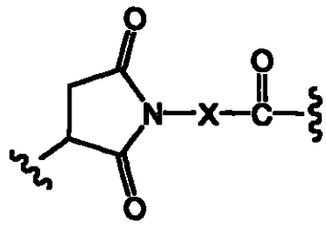
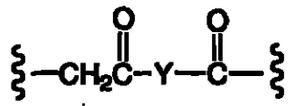
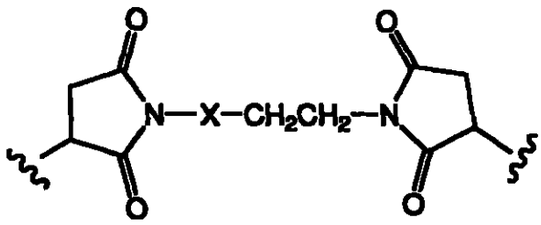
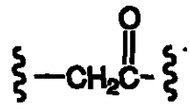
(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

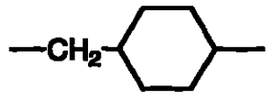
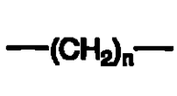
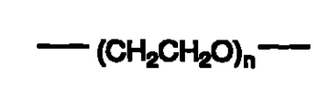
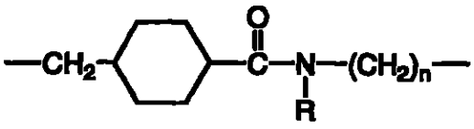
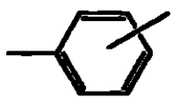
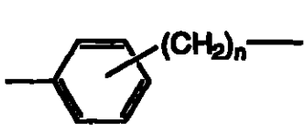
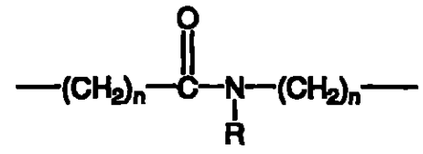
provided that the antibody is not TA.1.

L is a non-disulfide linker. L includes but is not limited to the structures:

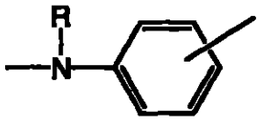
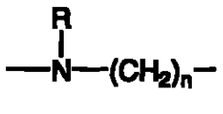





where the wavy lines indicate the covalent attachments to Ab and D;

X is:

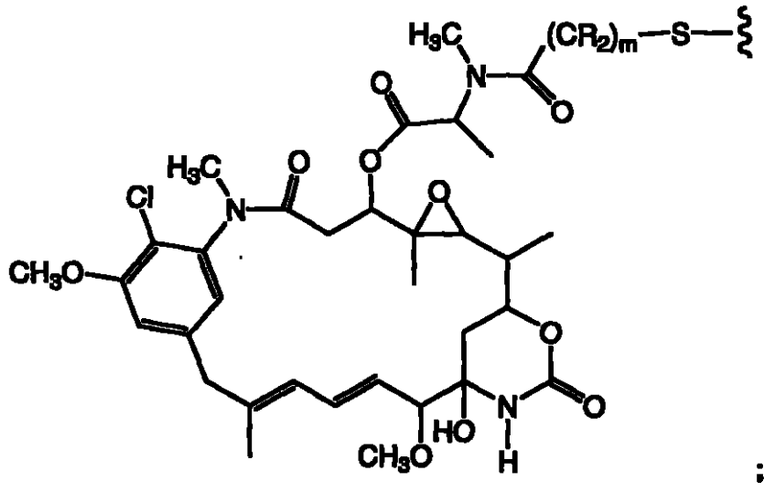








Y is:

R is independently H or C_1–C_6 alkyl; and n is 1 to 12;

D is a maytansinoid drug moiety. Maytansinoids include, but are not limited to the structure:



where the wavy line indicates the covalent attachment to L;

R is independently H or C_1–C_6 alkyl; and

m is 1, 2, or 3.

The drug to antibody ratio or drug loading is represented by p for Formula I compounds. The drug loading value p is 1 to 8. Formula I compounds include all mixtures of variously loaded and attached antibody-drug conjugates where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody.

In another embodiment, Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36), i.e. not to an ErbB receptor, including HER2.

ANTIBODIES

The antibody unit (Ab-) of Formula I includes within its scope any unit of an antibody that binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population. An antibody can be any protein or protein-like molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. In one aspect, the antibody unit acts to deliver the maytansinoid drug moiety to the particular target cell population with which the antibody unit reacts. Such antibodies include, but are not limited to, large molecular weight proteins such as, full-length antibodies and antibody fragments.

Antibodies comprising the antibody-drug conjugates of the invention preferably retain the antigen binding capability of their native, wild type counterparts. Thus, antibodies of the invention 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, 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). An antigen to which an antibody of the invention is capable of binding may be a member of a subset of one of the above-mentioned categories, wherein the other subset(s) of said category comprise other molecules/antigens that have a distinct characteristic (with respect to the antigen of interest).

In one embodiment, the antibody of the antibody-drug conjugates (ADC) specifically binds to a receptor encoded by an ErbB gene. The antibody may bind specifically to an ErbB receptor selected from EGFR, HER2, HER3 and HER4. The ADC may specifically bind to the extracellular domain (ECD) of the HER2 receptor and inhibit the growth of tumor cells which overexpress HER2 receptor. The antibody of the ADC may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanized antibody. A humanized antibody may be huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 or huMAb4D5-8 (trastuzumab). The antibody may be an antibody fragment, e.g. a Fab fragment.

Antibodies in Formula I antibody-drug conjugates (ADC) and which may be useful in the treatment of cancer include, but are not limited to, antibodies against cell surface receptors and tumor-associated antigens (TAA). Such tumor-associated antigens are known in the art, and can prepared for use in generating antibodies using methods and information which are well known in the art. In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Examples of TAA include, but are not limited to, Tumor-Associated Antigens (1)-(36) listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s), following nucleic acid and protein sequence identification conventions of the National Center for Biotechnology Information (NCBI). Nucleic acid and protein sequences corresponding to TAA (1)-(36) are available in public databases such as GenBank. Tumor-associated antigens targeted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the cited references, or which exhibit substantially the same biological properties or characteristics as a TAA having a sequence found in the cited references. For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure in the reference specifically recited herein are expressly incorporated by reference.

TUMOR-ASSOCIATED ANTIGENS (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203) ten Dijke,P., et al Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Claim 2); WO2003042661 (Claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (Claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (Claim 6); WO2003024392 (Claim 2; Fig 112); WO200298358 (Claim 1; Page 183); WO200254940 (Page 100-101); WO200259377(Page 349-350); WO200230268 (Claim 27; Page 376); WO200148204 (Example; Fig 4)

NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1 -

Cross-references: MIM:603248; NP_001194.1; AY065994

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486)
Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H.W., et al (1992) J. Biol. Chem. 267 (16):11267-11273); WO2004048938 (Example 2); WO2004032842 (Example IV); WO2003042661 (Claim 12); WO2003016475 (Claim 1); WO200278524 (Example 2); WO200299074 (Claim 19; Page 127-129); WO200286443 (Claim 27; Pages 222, 393); WO2003003906 (Claim 10; Page 293); WO200264798 (Claim 33; Page 93-95); WO200014228 (Claim 5; Page 133-136); US2003224454 (Fig 3); WO2003025138 (Claim 12; Page 150); US 20050107595; US 20050106644;
NP_003477 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 /pid=NP_003477.3 - Homo sapiens
Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449)
Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., et al (1999) Proc. Natl. Acad. Sci. U.S.A. 96 (25):14523-14528); WO2004065577 (Claim 6); WO2004027049 (Fig 1L); EP1394274 (Example 11); WO2004016225 (Claim 2); WO2003042661 (Claim 12); US2003157089 (Example 5); US2003185830 (Example 5); US2003064397 (Fig 2); WO200289747 (Example 5; Page 618-619); WO2003022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A);
NP_036581 six transmembrane epithelial antigen of the prostate
Cross-references: MIM:604415; NP_036581.1; NM_012449_1

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486)
J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Claim 14); WO200292836 (Claim 6; Fig 12); WO200283866 (Claim 15; Page 116-121); US2003124140 (Example 16); US2003091580 (Claim 6); WO200206317 (Claim 6; Page 400-408);
Cross-references: GI:34501467; AAK74120.3; AF361486_1

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823)
Yamaguchi, N., et al Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. U.S.A. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. U.S.A. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995)); WO2003101283 (Claim 14); (WO2002102235 (Claim 13; Page 287-288); WO2002101075 (Claim 4; Page 308-309); WO200271928 (Page 320-321); WO9410312 (Page 52-57);
Cross-references: MIM:601051; NP_005814.2; NM_005823_1

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b,Genbank accession no. NM_006424)
J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., et al (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582); WO2004022778 (Claim 2); EP1394274 (Example 11);

WO2002102235 (Claim 13; Page 326); EP875569 (Claim 1; Page 17-19); WO200157188 (Claim 20; Page 329); WO2004032842 (Example IV); WO200175177 (Claim 24; Page 139-140);
Cross-references: MIM:604217; NP_006415.1; NM_006424_1

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878)
Nagase T., et al (2000) DNA Res. 7 (2):143-150); WO2004000997 (Claim 1); WO2003003984 (Claim 1); WO200206339 (Claim 1; Page 50); WO200188133 (Claim 1; Page 41-43, 48-58); WO2003054152 (Claim 20); WO2003101400 (Claim 11);
Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628); Ross et al (2002) Cancer Res. 62:2546-2553; US2003129192 (Claim 2); US2004044180 (Claim 12); US2004044179 (Claim 11); US2003096961 (Claim 11); US2003232056 (Example 5); WO2003105758 (Claim 12); US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20);
Cross-references: GI:37182378; AAQ88991.1; AY358628_1

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
Nakamuta M., et al Biochem. Biophys. Res. Commun. 177, 34-39, 1991; Ogawa Y., et al Biochem. Biophys. Res. Commun. 178, 248-255, 1991; Arai H., et al Jpn. Circ. J. 56, 1303-1307, 1992; Arai H., et al J. Biol. Chem. 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., et al Biochem. Biophys. Res. Commun. 178, 656-663, 1991; Elshourbagy N.A., et al J. Biol. Chem. 268, 3873-3879, 1993; Haendler B., et al J. Cardiovasc. Pharmacol. 20, s1-S4, 1992; Tsutsumi M., et al Gene 228, 43-49, 1999; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; Bourgeois C., et al J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., et al Biol. Chem. 272, 21589-21596, 1997; Verheij J.B., et al Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R.M.W., et al Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E.G., et al Cell 79, 1257-1266, 1994; Attie T., et al, Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., et al Hum. Mol. Genet. 5:351-354, 1996; Amiel J., et al Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R.M.W., et al Nat. Genet. 12, 445-447, 1996; Svensson P.J., et al Hum. Genet. 103, 145-148, 1998; Fuchs S., et al Mol. Med. 7, 115-124, 2001; Pingault V., et al (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1); WO2004048938 (Example 2); WO2004040000 (Claim 151); WO2003087768 (Claim 1); WO2003016475 (Claim 1); WO2003016475 (Claim 1); WO200261087 (Fig 1); WO2003016494 (Fig 6); WO2003025138 (Claim 12; Page 144); WO200198351 (Claim 1; Page 124-125); EP522868 (Claim 8; Fig 2); WO200177172 (Claim 1; Page 297-299); US2003109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004001004;

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

WO2003104275 (Claim 1); WO2004046342 (Example 2); WO2003042661 (Claim 12); WO2003083074 (Claim 14; Page 61); WO2003018621 (Claim 1); WO2003024392 (Claim 2; Fig 93); WO200166689 (Example 6);
Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138)
Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (Claim 1; Fig 1); WO200272596 (Claim 13; Page 54-55); WO200172962 (Claim 1; Fig 4B); WO2003104270 (Claim 11); WO2003104270 (Claim 16); US2004005598 (Claim 22); WO2003042661 (Claim 12); US2003060612 (Claim 12; Fig 10); WO200226822 (Claim 23; Fig 2); WO200216429 (Claim 12; Fig 10);
Cross-references: GI:22655488; AAN04080.1; AF455138_1

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636)
Xu, X.Z., et al Proc. Natl. Acad. Sci. U.S.A. 98 (19):10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33):30813-30820 (2003)); US2003143557 (Claim 4); WO200040614 (Claim 14; Page 100-103); WO200210382 (Claim 1; Fig 9A); WO2003042661 (Claim 12); WO200230268 (Claim 27; Page 391); US2003219806 (Claim 4); WO200162794 (Claim 14; Fig 1A-D);
Cross-references: MIM:606936; NP_060106.2; NM_017636_1

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212)
Ciccodicola, A., et al EMBO J. 8 (7):1987-1991 (1989), Am. J. Hum. Genet. 49 (3):555-565 (1991)); US2003224411 (Claim 1); WO2003083041 (Example 1); WO2003034984 (Claim 12); WO200288170 (Claim 2; Page 52-53); WO2003024392 (Claim 2; Fig 58); WO200216413 (Claim 1; Page 94-95, 105); WO200222808 (Claim 2; Fig 1); US5854399 (Example 2; Col 17-18); US5792616 (Fig 2);
Cross-references: MIM:187395; NP_003203.1; NM_003212_1

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004)
Fujisaku et al (1989) J. Biol. Chem. 264 (4):2118-2125); Weis J.J., et al J. Exp. Med. 167, 1047-1066, 1988; Moore M., et al Proc. Natl. Acad. Sci. U.S.A. 84, 9194-9198, 1987; Barel M., et al Mol. Immunol. 35, 1025-1031, 1998; Weis J.J., et al Proc. Natl. Acad. Sci. U.S.A. 83, 5639-5643, 1986; Sinha S.K., et al (1993) J. Immunol. 150, 5311-5320; WO2004045520 (Example 4); US2004005538 (Example 1); WO2003062401 (Claim 9); WO2004045520 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Claim 1);
Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626 or 11038674)
Proc. Natl. Acad. Sci. U.S.A. (2003) 100 (7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller et al (1992) Eur. J. Immunol. 22 (6):1621-1625); WO2004016225 (claim 2, Fig 140); WO2003087768, US2004101874 (claim 1, page 102); WO2003062401 (claim 9); WO200278524 (Example 2); US2002150573 (claim 5, page 15); US5644033; WO2003048202 (claim 1, pages 306 and 309); WO 99/558658, US6534482 (claim 13, Fig 17A/B); WO200055351 (claim 11, pages 1145-1146);
Cross-references: MIM:147245; NP_000617.1; NM_000626_1

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764, AY358130)
Genome Res. 13 (10):2265-2270 (2003), Immunogenetics 54 (2):87-95 (2002), Blood 99 (8):2662-2669 (2002), Proc. Natl. Acad. Sci. U.S.A. 98 (17):9772-9777 (2001), Xu, M.J., et al (2001) Biochem. Biophys. Res. Commun. 280 (3):768-775; WO2004016225 (Claim 2); WO2003077836; WO200138490 (Claim 5; Fig 18D-1-18D-2); WO2003097803 (Claim 12); WO2003089624 (Claim 25);
Cross-references: MIM:606509; NP_110391.2; NM_030764_1

(17) HER2 (ErbB2, Genbank accession no. M11730)
Coussens L., et al Science (1985) 230(4730):1132-1139); Yamamoto T., et al Nature 319, 230-234, 1986; Semba K., et al Proc. Natl. Acad. Sci. U.S.A. 82, 6497-6501, 1985; Swiercz J.M., et al J. Cell Biol. 165, 869-880, 2004; Kuhns J.J., et al J. Biol. Chem. 274, 36422-36427, 1999; Cho H.-S., et al Nature 421, 756-760, 2003; Ehsani A., et al (1993) Genomics 15, 426-429; WO2004048938 (Example 2); WO2004027049 (Fig 1I); WO2004009622; WO2003081210; WO2003089904 (Claim 9); WO2003016475 (Claim 1); US2003118592; WO2003008537 (Claim 1); WO2003055439 (Claim 29; Fig 1A-B); WO2003025228 (Claim 37; Fig 5C); WO200222636 (Example 13; Page 95-107); WO200212341 (Claim 68; Fig 7); WO200213847 (Page 71-74); WO200214503 (Page 114-117); WO200153463 (Claim 2; Page 41-46); WO200141787 (Page 15); WO200044899 (Claim 52; Fig 7); WO200020579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4);
Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

(18) NCA (CEACAM6, Genbank accession no. M18728);
Barnett T., et al Genomics 3, 59-66, 1988; Tawaragi Y., et al Biochem. Biophys. Res. Commun. 150, 89-96, 1988; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903, 2002; WO2004063709; EP1439393 (Claim 7); WO2004044178 (Example 4); WO2004031238; WO2003042661 (Claim 12); WO200278524 (Example 2); WO200286443 (Claim 27; Page 427); WO200260317 (Claim 2);
Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;

(19) MDP (DPEP1, Genbank accession no. BC017023)

Proc. Natl. Acad. Sci. U.S.A. 99 (26):16899-16903 (2002)); WO2003016475 (Claim 1); WO200264798 (Claim 33; Page 85-87); JP05003790 (Fig 6-8); WO9946284 (Fig 9); Cross-references: MIM:179780; AAH17023.1; BC017023_1

(20) IL20Rα (IL20Ra, ZCYTOR7, Genbank accession no. AF184971); Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Mungall A.J., et al Nature 425, 805-811, 2003; Blumberg H., et al Cell 104, 9-19, 2001; Dumoutier L., et al J. Immunol. 167, 3545-3549, 2001; Parrish-Novak J., et al J. Biol. Chem. 277, 47517-47523, 2002; Pletnev S., et al (2003) Biochemistry 42:12617-12624; Sheikh F., et al (2004) J. Immunol. 172, 2006-2010; EP1394274 (Example 11); US2004005320 (Example 5); WO2003029262 (Page 74-75); WO2003002717 (Claim 2; Page 63); WO200222153 (Page 45-47); US2002042366 (Page 20-21); WO200146261 (Page 57-59); WO200146232 (Page 63-65); WO9837193 (Claim 1; Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) Brevican (BCAN, BEHAB, Genbank accession no. AF229053) Gary S.C., et al Gene 256, 139-147, 2000; Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Stransberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; US2003186372 (Claim 11); US2003186373 (Claim 11); US2003119131 (Claim 1; Fig 52); US2003119122 (Claim 1; Fig 52); US2003119126 (Claim 1); US2003119121 (Claim 1; Fig 52); US2003119129 (Claim 1); US2003119130 (Claim 1); US2003119128 (Claim 1; Fig 52); US2003119125 (Claim 1); WO2003016475 (Claim 1); WO200202634 (Claim 1);

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442) Chan,J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000)); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42); Cross-references: MIM:600997; NP_004433.2; NM_004442_1

(23) ASLG659 (B7h, Genbank accession no. AX092328) US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); US2003165504 (Claim 1); US2003124140 (Example 2); US2003065143 (Fig 60); WO2002102235 (Claim 13; Page 299); US2003091580 (Example 2); WO200210187 (Claim 6; Fig 10); WO200194641 (Claim 12; Fig 7b); WO200202624 (Claim 13; Fig 1A-1B); US2002034749 (Claim 54; Page 45-46); WO200206317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587 (Example 1; Fig 1); WO200140269 (Example 3; Pages 190-192); WO200036107 (Example 2; Page 205-207); WO2004053079 (Claim 12); WO2003004989 (Claim 1); WO200271928 (Page 233-234, 452-453); WO 0116318;

(24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436)

Reiter R.E., et al Proc. Natl. Acad. Sci. U.S.A. 95, 1735-1740, 1998; Gu Z., et al Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-788; WO2004022709; EP1394274 (Example 11); US2004018553 (Claim 17); WO2003008537 (Claim 1); WO200281646 (Claim 1; Page 164); WO2003003906 (Claim 10; Page 288); WO200140309 (Example 1; Fig 17); US2001055751 (Example 1; Fig 1b); WO200032752 (Claim 18; Fig 1); WO9851805 (Claim 17; Page 97); WO9851824 (Claim 10; Page 94); WO9840403 (Claim 2; Fig 1B); Accession: O43653; EMBL; AF043498; AAC39607.1.

(25) GEDA (Genbank accession No. AY260763); AAP14954 lipoma HMGIC fusion-partner-like protein /pid=AAP14954.1 - Homo sapiens Species: Homo sapiens (human) WO2003054152 (Claim 20); WO2003000842 (Claim 1); WO2003023013 (Example 3, Claim 20); US2003194704 (Claim 45); Cross-references: GI:30102449; AAP14954.1; AY260763_1

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, Genbank accession No. AF116456); BAFF receptor /pid=NP_443177.1 - Homo sapiens Thompson, J.S., et al Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611; WO2003045422 (Example; Page 32-33); WO2003014294 (Claim 35; Fig 6B); WO2003035846 (Claim 70; Page 615-616); WO200294852 (Col 136-137); WO200238766 (Claim 3; Page 133); WO200224909 (Example 3; Fig 3); Cross-references: MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814, Genbank accession No. AK026467); Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Claim 1; Fig 1); Cross-references: MIM:107266; NP_001762.1; NM_001771_1

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation) PROTHIN SEQUENCE Full mpggpgv...dvqlekp (1..226; 226 aa), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.10) WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4, pages 13-14); WO9958658 (claim 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha et al (1992) J. Immunol. 148(5):1526-1531; Mueller et al (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et al (1994) Immunogenetics 40(4):287-295; Preud'homme et al (1992) Clin. Exp. Immunol. 90(1):141-146; Yu et al (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al (1988) EMBO J. 7(11):3457-3464;

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and

perhaps development of AIDS, lymphoma, myeloma, and leukemia) PROTEIN SEQUENCE Full mnyplti...atsltf (1..372; 372 aa), pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No. NP_001707.1)
WO2004040000; WO2004015426; US2003105292 (Example 2); US6555339 (Example 2); WO200261087 (Fig 1); WO200157188 (Claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7, Fig 5); Dobner et al (1992) Eur. J. Immunol. 22:2795-2799; Barella et al (1995) Biochem. J. 309:773-779;

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes) PROTEIN SEQUENCE Full mgagwvp...vllpqac (1..273; 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1)
Tonnelle et al (1985) EMBO J. 4(11):2839-2847; Jonsson et al (1989) Immunogenetics 29(6):411-413; Beck et al (1992) J. Mol. Biol. 228:433-441; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al (1987) J. Biol. Chem. 262:8759-8766; Beck et al (1996) J. Mol. Biol. 255:1-13; Naruse et al (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-146); Kasahara et al (1989) Immunogenetics 30(1):66-68; Larhammar et al (1985) J. Biol. Chem. 260(26):14111-14119;

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability) PROTEIN SEQUENCE Full mgqagck...lephrst (1..422; 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2)
Le et al (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman et al (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, Genbank accession No. NP_001773.1)
WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-106); Von Hoegen et al (1990) J. Immunol. 144(12):4870-4877; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis) PROTEIN SEQUENCE Full mafdvsc...rwkyqhi (1..661; 661 aa), pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1)
US2002193567; WO9707198 (claim 11, pages 39-42); Miura et al (1996) Genomics 38(3):299-304; Miura et al (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-61); WO200012130 (pages 24-26);

(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation) PROTEIN SEQUENCE Full mlprlll...vdyedam (1..429; 429 aa), pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, Genbank accession No. NP_443170.1)
WO2003077836; WO200138490 (claim 6, Fig 18B-1-18-E-2); Davis et al (2001) Proc. Natl. Acad. Sci USA 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1); WO2003089624 (claim 7);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies) PROTEIN SEQUENCE Full mllwvil...eaaaphr (1..977; 977 aa), pI: 6.88 MW: 106468 TM: 1 [P] Gene Chromosome: 1q21, Genbank accession No. Human:AF343662, AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse:AK089756, AY158090, AY506558; NP_112571.1
WO2003024392 (claim 2, Fig 97); Nakayama et al (2000) Biochem. Biophys. Res. Commun. 277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2);

(36) TENB2 (TMEFF2, tomoregulin, TPEF, HPP1, TR, putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin) PROTEIN SEQUENCE Full mvlwsap...rastrli (1..374; 374 aa, NCBI Accession: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; Genbank accession No. AF179274; AY358907, CAF85723, CQ782436
WO2004074320 (SEQ ID NO 810); JP2004113151 (SEQ ID NOS 2, 4, 8); WO2003042661 (SEQ ID NO 580); WO2003009814 (SEQ ID NO 411); EP1295944 (pages 69-70); WO200230268 (page 329); WO200190304 (SEQ ID NO 2706); US2004249130; US2004022727; WO2004063355; US2004197325; US2003232350; US2004005563; US2003124579; US 6410506; US 6642006l; Horie et al (2000) Genomics 67:146-152; Uchida et al (1999) Biochem. Biophys. Res. Commun. 266:593-602; Liang et al (2000) Cancer Res. 60:4907-12; Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84.

PRODUCTION OF ANTIBODIES

Various methods have been employed to produce monoclonal antibodies (MAbs). Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Other methods to prepare MAbs, including chimeric and humanized antibodies, uses genetic engineering, i.e. recombinant DNA techniques.

Polyclonal antibodies may be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, (1984) J. Immunol., 133:3001, and Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York,

1987)). Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al (1980) Anal. Biochem. 107:220.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells (US 2005/0048572; US 2004/0229310). Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al (1993) Curr. Opinion in Immunol. 5:256-262 and Plückthun (1992) Immunol. Revs. 130:151-188.

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

The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (US 4816567); and Morrison et al (1984) Proc. Natl Acad. Sci. USA 81:6851), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

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

A description follows as to exemplary techniques for the production of the antibodies (Ab) used in the antibody-drug conjugates (ADC) of the present invention. The production of antibodies will be illustrated with reference to anti-ErbB2 antibodies but it will be apparent for those skilled in the art that antibodies to other members of the ErbB receptor family, as well as any other receptor or tumor-associated antigen or target, can be produced and modified in a similar manner.

The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface, e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma

cell line such as SK-BR-3 cells (Stancovski et al (1991) PNAS (USA) 88:8691-8695), can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

Example 1 describes production of an exemplary humanized anti-ErbB2 antibody. The humanized antibody may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth 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, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H.

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production (Jakobovits et al (1993) Proc. Natl. Acad. Sci. USA, 90:2551; Jakobovits et al (1993) Nature 362:255-258; Bruggermann et al (1993) Year in Immuno. 7:33; and US 5591669; US 5589369; US 5545807).

Alternatively, phage display technology (McCafferty et al (1990) Nature 348:552-553) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors (Johnson, Kevin S. and Chiswell, David J. (1993) *Current Opinion in Structural Biology* 3:564-571). A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially (Marks et al (1991) J. Mol. Biol. 222:581-597; Griffith et al (1993) EMBO J. 12:725-734; US 5565332; US 5573905). Human antibodies may also be generated by *in vitro* activated B cells (US 5567610; US 5229275). Human anti-ErbB2 antibodies are described (US 5772997 and WO 97/00271.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see Morimoto et al (1992) Journal of Biochemical and Biophysical Methods 24:107-117; and Brennan et al (1985) Science 229:81). Antibody fragments can also be produced directly by recombinant host cells and the antibody phage libraries discussed above. 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). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; US 5571894; and US 5587458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US 5641870 for example. Such linear antibody fragments may be monospecific or bispecific.

Bispecific antibodies with binding specificities for at least two different epitopes (Millstein et al (1983), Nature 305:537-539) may bind to two different epitopes of the ErbB2 protein. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2 (WO 96/16673; US

5837234; WO98/02463; US 5821337). Purification methods for bispecific antibodies have been disclosed (WO 93/08829; Traunecker et al (1991) EMBO J. 10:3655-3659; WO 94/04690; Suresh et al (1986) *Methods in Enzymology* 121:210; US 5731168). Bispecific antibodies can be produced using leucine zippers (Kostelny et al (1992) J. Immunol. 148(5):1547-1553), and single-chain Fv (sFv) dimers (Gruber et al (1994) J. Immunol. 152:5368).

Techniques for generating bispecific antibodies from antibody fragments have also been described, such as using chemical linkage wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments (Brennan et al (1985) Science 229:81). Fab'-SH fragments can be recovered from *E. coli* and chemically coupled to form bispecific antibodies (Shalaby et al (1992) J. Exp. Med. 175:217-225. The "diabody" technology provides an alternative method for making bispecific antibody fragments (Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448).

Antibodies with more than two valencies are contemplated. Multivalent, "Octopus" antibodies with three or more antigen binding sites and two or more variable domains can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody (US 2002/0004586; WO 01/77342). For example, trispecific antibodies can be prepared (Tutt et al (1991) J. Immunol. 147:60.

Amino acid sequence modification(s) of antibodies are contemplated. For example, mutants and various isoforms of antibodies which bind to tumor-associated antigens are contemplated to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody are prepared by introducing appropriate nucleotide changes into the nucleic acid 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 is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is "alanine scanning mutagenesis" (Cunningham and Wells (1989) Science 244:1081-1085) where an amino acid 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, such as alanine or polyalanine, to optimize the interaction of the amino acids with antigen. 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 anti-ErbB2 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-ErbB2 antibody molecule include the fusion to the N- or C-terminus of the anti-ErbB2 antibody to an enzyme (e.g. for ADEPT: Tietze et al (2003) Current Pharm. Design 9:2155-2175) or a polypeptide which increases the serum half-life of the antibody, such as an albumin-binding peptide.

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, SEQ ID NOS: z1-z14, and all of which are incorporated herein by reference.

The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. 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. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

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. Naturally occurring residues are divided into groups based on common side-chain properties:

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

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

(3) acidic: asp, glu;

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

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

(6) aromatic: trp, tyr, phe.

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

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

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., IgG_1, IgG_2, IgG_3, or IgG_4) 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 5834597).

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), or the extent of glycosylation.

Antibodies may be glycosylated at conserved positions (N-linked or O-linked) in their constant regions (Hse et al (1997) J. Biol. Chem. 272:9062-9070; Jefferis and Lund, (1997) Chem. Immunol. 65:111-128; Wright and Morrison, (1997) TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al (1996) Mol. Immunol. 32:1311-1318; Wittwe and Howard, (1990) Biochem. 29:4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner (1996) Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures (Malhotra et al (1995) Nature Med. 1:237-243; Umana et al (1999) Nature Biotech. 17:176-180). Removal of the oligosaccharides may optimize antigen binding and other properties of the antibody (Boyd et al (1996) Mol. Immunol. 32:1311-1318).

Factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like (US 5047335; US 5510261; US 5278299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

MAYTANSINOID DRUG MOIETIES

Maytansine compounds inhibit cell proliferation by inhibiting the formation of microtubules during mitosis through inhibition of polymerization of the microtubulin protein, tubulin (Remillard et al (1975) Science 189:1002-1005; US 5208020). Maytansine and maytansinoids are highly cytotoxic but their clinical use in cancer therapy has been greatly limited by their severe systemic side-effects primarily attributed to their poor selectivity for tumors. Clinical trials with maytansine had been discontinued due to serious adverse effects on the central nervous system and gastrointestinal system (Issel et al (1978) Can. Treatment. Rev. 5:199-207.

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.

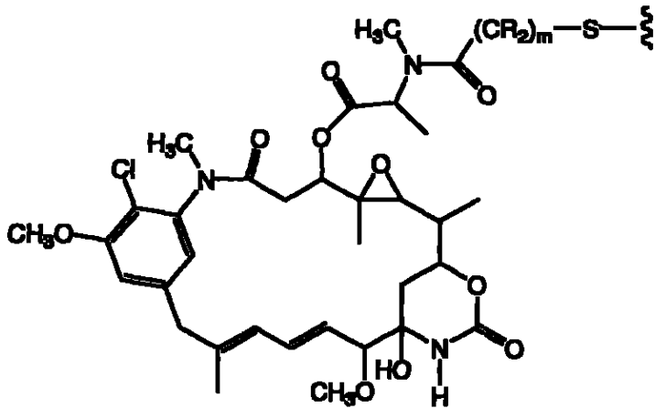
Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared 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 ansamitocin 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 (US 4294757) (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, prepared by the reaction of maytansinol with H_2S or P_2S_5 (US 4424219); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) prepared from *Nocardia* (US 4450254); C-15-hydroxy/acyloxy, prepared by the conversion of maytansinol by Streptomyces (US 4364866); C-15-methoxy, isolated from Trewia nudiflora (US 4313946 and US 4315929); C-18-N-demethyl, prepared by the demethylation of maytansinol by Streptomyces (US 4362663 and US 4322348); and 4,5-deoxy, prepared by the titanium trichloride/LAH reduction of maytansinol (US 4371533).

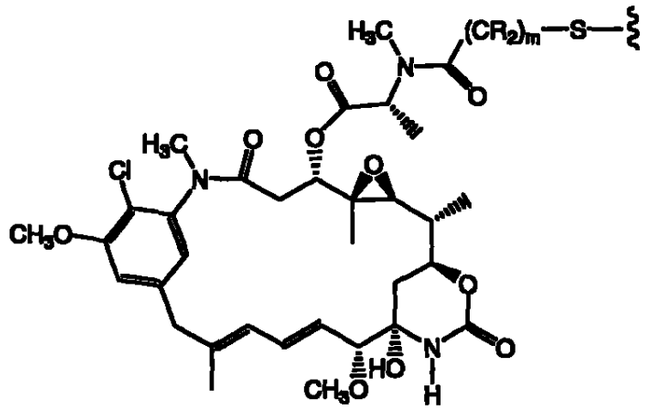
Many positions on maytansine compounds are known to be useful as the linkage position, depending upon the type of link. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group and the C-20 position having a hydroxyl group are all suitable.

Maytansinoid drug moieties (D) include those having the structure:



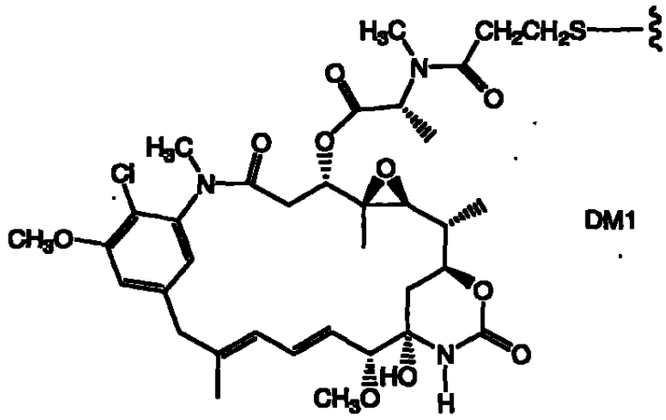
where the wavy line indicates the covalent attachment of the sulfur atom of D to a linker (L) of an antibody-drug conjugate (ADC). R may independently be H or a C_1–C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e. m is 1, 2, or 3 (US 633410, 5208020, Chari et al (1992) Cancer Res. 52:127-131; Liu et al (1996) Proc. Natl. Acad. Sci 93:8618-8623).

All stereoisomers of the maytansinoid drug moiety are contemplated for the compounds of the invention, i.e. any combination of *R* and *S* configurations at the chiral carbons of D. In one embodiment, the maytansinoid drug moiety (D) will have the following stereochemistry:



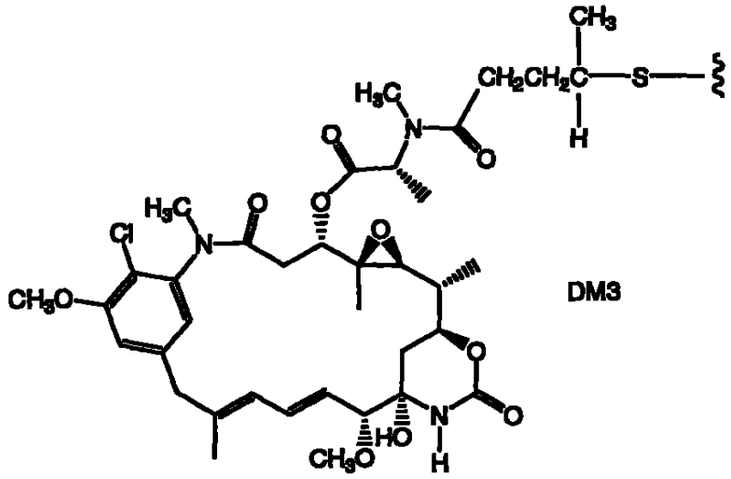
Embodiments of D include:

DM1 ($N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) where $(CR_2)_m = CH_2CH_2$;



DM1

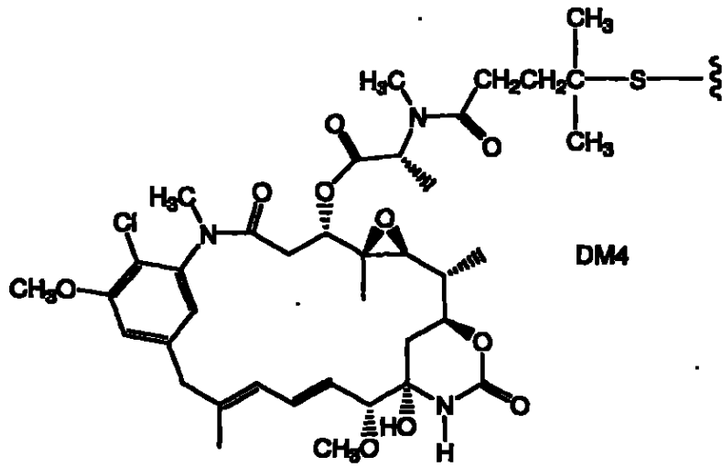
DM3 ($N^{2'}$-deacetyl-$N^{2'}$(4-mercapto-1-oxopentyl)-maytansine) where $(CR_2)_m = CH_2CH_2CH(CH_3)$;



DM3

DM4 ($N^{2'}$-deacetyl-$N^{2'}$(4-methyl-4-mercapto-1-oxopentyl)-maytansine) where $(CR_2)_m$ =

$CH_2CH_2C(CH_3)_2$:



Steric hindrance conferred by alkyl groups such as the methyl groups on the carbon adjacent to the sulfur atom of DM3 and DM4 may affect the rate of intracellular cleavage of the ADC (US 2004/0235840 A1). The variable alkyl unit $(CR_2)_m$ may therefore affect potency, efficacy, and safety/toxicity in vitro and in vivo.

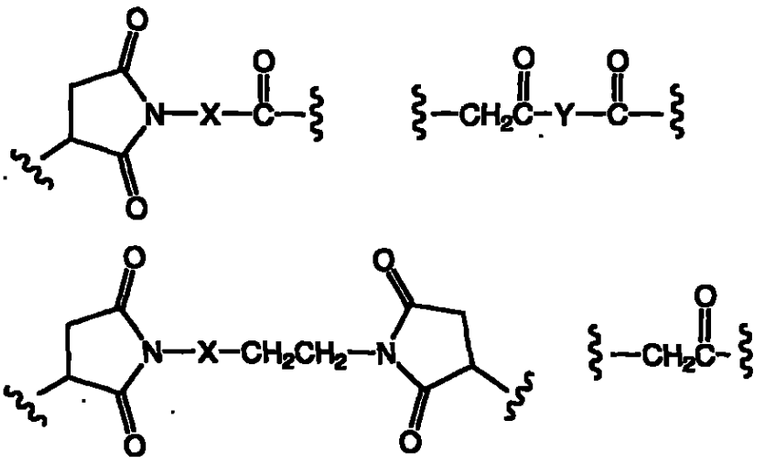
LINKERS

The linker, L, attaches the antibody to a drug moiety through covalent bond(s), not comprising a disulfide group. The linker 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, or an amine, e.g. N-terminus or amino acid side chain such as lysine, of the antibody (Ab) can form a bond with a functional group of a linker reagent, drug moiety or drug-linker reagent.

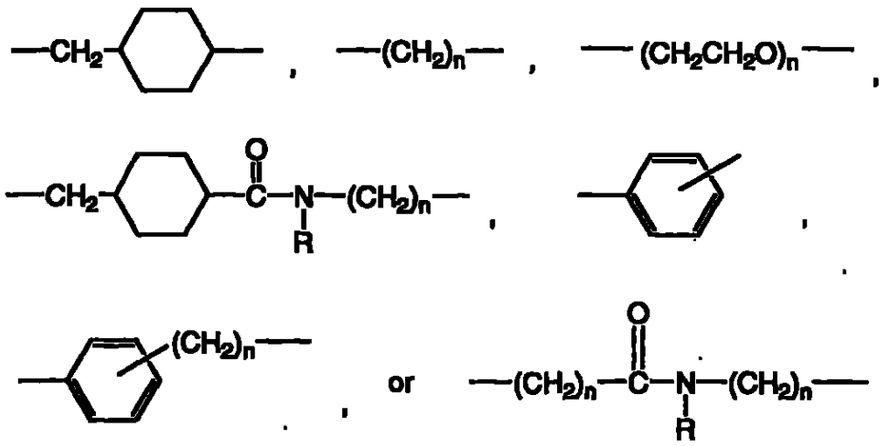
The linkers are preferably stable extracellularly. Before transport or delivery into a cell, the antibody-drug conjugate (ADC) is preferably stable and remains intact, i.e. the antibody remains linked to the drug moiety. The linkers are stable outside the target cell and may be cleaved at some efficacious rate inside the cell. An effective linker will: (i) maintain the specific binding properties of the antibody; (ii) allow intracellular delivery of the conjugate or drug moiety; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its targetted site; and (iv) maintain a cytotoxic, cell-killing effect or a cytostatic effect of the maytansinoid drug moiety. Stability of the ADC may be measured by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS.

Covalent attachment of the antibody and the drug moiety requires the linker to have two reactive functional groups, i.e. bivalency in a reactive sense. Bivalent linker reagents which are useful to attach two or more functional or biologically active moieties, such as peptides, nucleic acids, drugs, toxins, antibodies, haptens, and reporter groups are known, and methods have been described their resulting conjugates (Hermanson, G.T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p234-242).

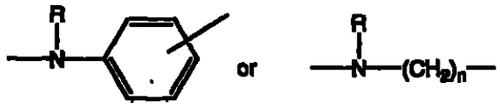
Linkers may have structures selected from:



where the wavy lines indicate the covalent attachments to Ab and D in either orientation. X may have the structures, in either orientation:

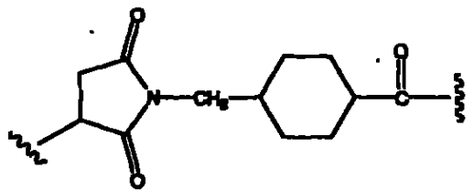


where R is independently H or C_1–C_6 alkyl; and n is 1 to 12. Y may have the structures, in either orientation:

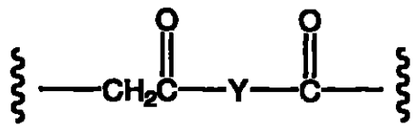


where R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

For example, the linker may have the structure, designated as SMCC:

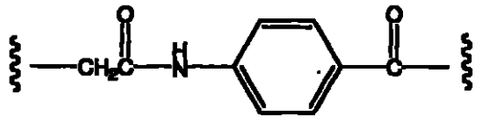


In another embodiment, linker (L) has the structure:

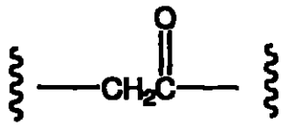


where the wavy lines indicate the covalent attachments to Ab and D in either orientation.

For example, the linker may have the structure, designated as SIAB:



In another embodiment, linker (L) has the structure:



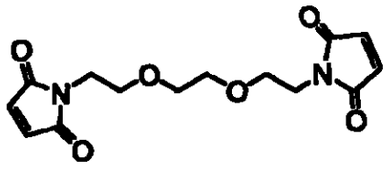
In another embodiment, the linker may be substituted with groups which modulated solubility or reactivity. For example, a sulfonate substituent may increase water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody or the drug moiety, or facilitate the coupling reaction of Ab-L with D, or D-L with Ab, depending on the synthetic route employed to prepare the ADC.

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.

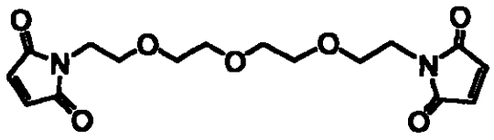
Linkers can be peptidic, comprising one or more amino acid units. Peptide linker reagents may be prepared by solid phase or liquid phase synthesis methods (E. Schröder and K. Lübke, *The Peptides*, volume 1, pp 76-136 (1965) Academic Press) that are well known in the field of peptide chemistry, including t-BOC chemistry (Geiser et al "Automation of solid-phase peptide synthesis" in *Macromolecular Sequencing and Synthesis*, Alan R. Liss, Inc., 1988, pp. 199-218) and Fmoc/HBTU chemistry (Fields, G. and Noble, R. (1990) "Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids", Int. J. Peptide Protein Res. 35:161-214), on an automated synthesizer such as the Rainin Symphony Peptide Synthesizer (Protein Technologies, Inc., Tucson, AZ), or Model 433 (Applied Biosystems, Foster City, CA).

The compounds expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPEO, 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), 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. See pages 467-498, 2003-2004 Applications Handbook and Catalog. Bis-maleimide reagents allow the attachment of a free thiol group of a cysteine residue of an 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 an antibody, maytansinoid drug moiety, or linker intermediate include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

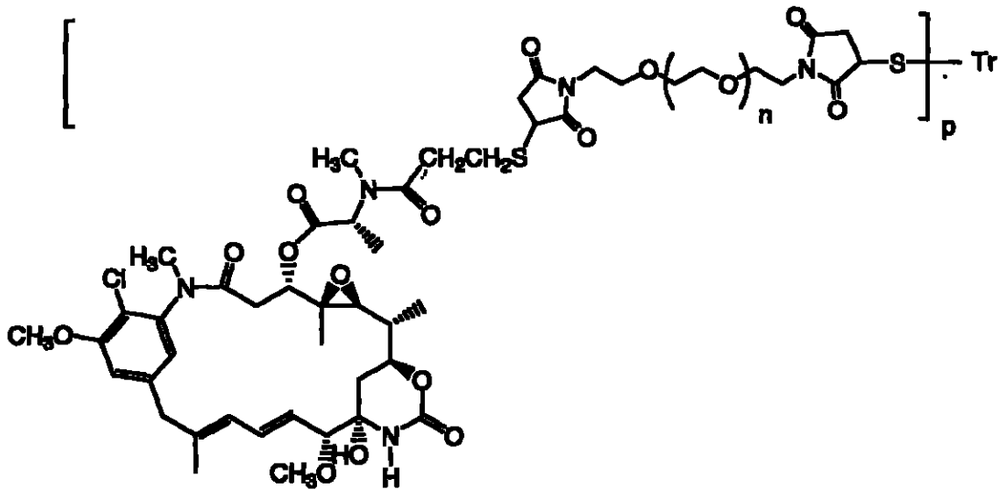


BM(PEO)$_3$



BM(PEO)$_4$

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

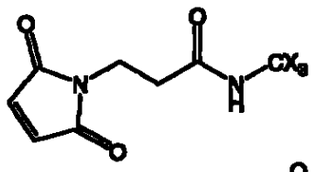


where Tr is trastuzumab; n is 0, 1, or 2; and p is 1, 2, 3, or 4.

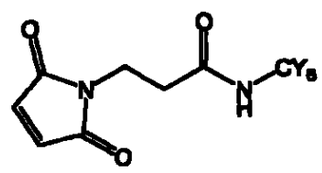
Useful linker reagents can also be obtained via other commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or synthesized in accordance with procedures described in Toki et al (2002) J. Org. Chem. 67:1866-1872; US 6214345 to Firestone et al; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

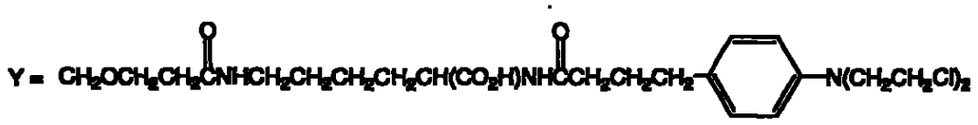
The Linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to an antibody (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768; King et al (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 an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

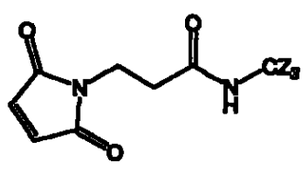
The following exemplary embodiments of dendritic linker reagents allow up to nine nucleophilic drug moiety reagents to be conjugated by reaction with the chloroethyl nitrogen mustard functional groups:





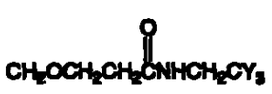








or



DRUG LOADING

The drug loading is represented by p in a molecule of Formula I, the average number of maytansinoid drugs per antibody. Drug loading may range from 1 to 8 drugs (D) per antibody (Ab), i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody. Compositions of ADC of Formula I include collections of antibodies conjugated with a range of drugs, from 1 to 8. The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, electrophoresis, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clinical Cancer Res. 10:7063-7070; Sanderson et al (2005) Clinical Cancer Res. 11:843-852). However, the distribution of p (drug) values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also, ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. 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. Higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the drug-linker intermediate (D-L) or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. Additionally, the antibody must be 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 several different manners, including: (i) limiting the molar excess of drug-linker intermediate (D-L) or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

Where more than one nucleophilic or electrophilic group of the antibody 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 drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (p) may be isolated ("Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate", Hamblett, K.J., et al, Abstract No. 624, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; "Controlling the Location of Drug Attachment in Antibody-Drug Conjugates", Alley, S.C., et al, Abstract No. 627, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). However, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody.

PREPARATION OF 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 nucleophilic group or an electrophilic group of an antibody with a bivalent 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 or an electrophilic group of a drug moiety with a linker reagent, to form drug-linker intermediate D-L, via a covalent bond, followed by reaction with the nucleophilic group or an electrophilic group of an antibody. Conjugation methods (1) and (2) may be employed with a variety of antibodies, drug moieties, and linkers to prepare the antibody-drug conjugates of Formula I.

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 (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). Each cysteine disulfide 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.

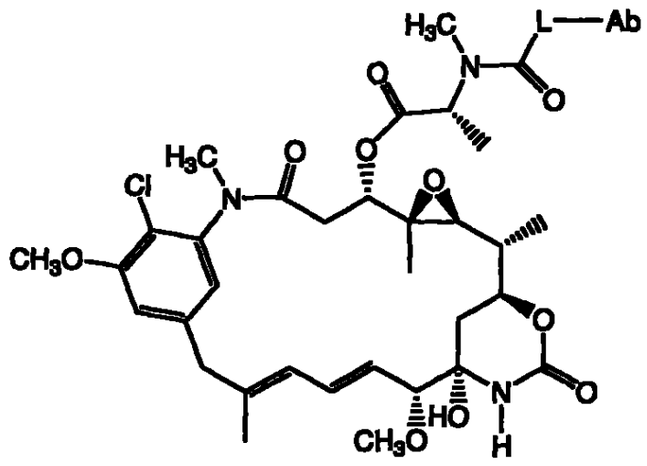
Antibody-drug conjugates may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic substituents on the linker reagent or drug. The sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde 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 galactose 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, G.T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p234-242). 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.

Maytansine may, for example, be converted to May-$SSCH_3$, which can be reduced to the free thiol, May-SH, and reacted with a modified antibody (Chari et al (1992) Cancer Research 52:127-131) to generate a maytansinoid-antibody immunoconjugate with a disulfide linker. Antibody-maytansinoid conjugates with disulfide linkers have been reported (WO 04/016801; US 6884874; US 2004/039176 A1; WO 03/068144; US 2004/001838 A1; US 6441163; US 5208020; US 5416064; WO 01/024763). The disulfide linker SPP is constructed with linker reagent N-succinimidyl 4-(2-pyridylthio) pentanoate. Antibody-SPP-DM1 conjugates are represented by the structure:

Disulfide (S-S) linker antibody-drug conjugates were tested for purposes of comparison to the non-disulfide linker ADC of the invention. Trastuzumab-SPP-DM1 was prepared according to Example 3 (Ranson, M. and Sliwkowski M. (2002) Oncology 63(suppl 1):17-24). Disulfide antibody-drug conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM3, and trastuzumab-SPP-DM4 were also tested, and have the structures

below:

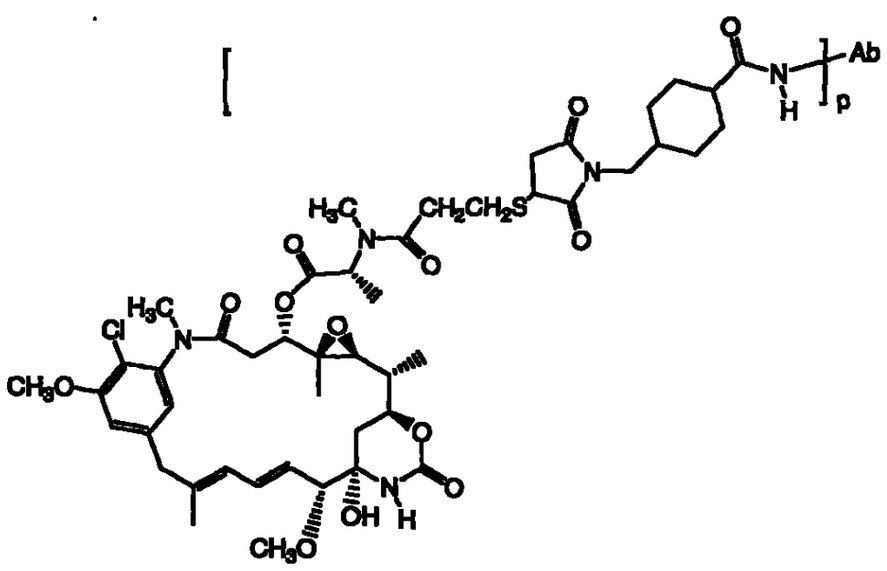


L—Ab = CH_2CH_2-S-S-CH_2CH_2C(=O)NH—Tr Trastuzumab-SPDP-DM1

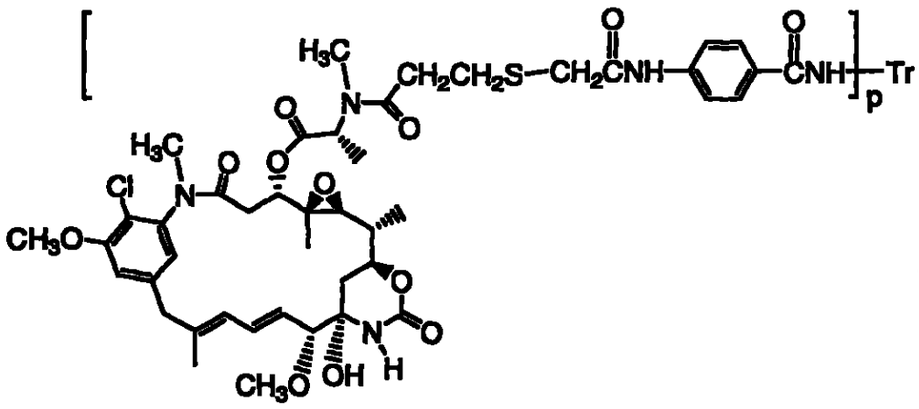
L—Ab = $CH_2CH(CH_3)$-S-S-$CH(CH_3)CH_2CH_2$C(=O)NH—Tr Trastuzumab-SPP-DM3

L—Ab = $CH_2C(CH_3)_2$-S-S-$CH(CH_3)CH_2CH_2$C(=O)NH—Tr Trastuzumab-SPP-DM4

ADC of the invention include SMCC linkers and the DM1 maytansinoid drug moiety, represented as Ab-SMCC-DM1:



One embodiment of Ab-SMCC-DM1 is trastuzumab-SMCC-DM1 where p is 1, 2, 3, or 4 (Ab = trastuzumab, Tr, WO 2005/037992). Another embodiment of an ADC is trastuzumab-SIAB-DM1 (trastuzumab = Tr) having the structure:



SCREENING FOR ANTIBODY-DRUG CONJUGATES (ADC) DIRECTED AGAINST TUMOR-ASSOCIATED ANTIGENS AND CELL SURFACE RECEPTORS

Transgenic animals and cell lines are particularly useful in screening antibody-drug conjugates (ADC) that have potential as prophylactic or therapeutic treatments of diseases or disorders involving overexpression of tumor-associated antigens and cell surface receptors, e.g. HER2 (US 6632979). Screening for a useful ADC may involve administering candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. Candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format. The rate at which ADC may be screened for utility for prophylactic or therapeutic treatments of diseases or disorders is limited only by the rate of synthesis or screening methodology, including detecting/measuring/analysis of data.

One embodiment is a screening method comprising (a) transplanting cells from a stable breast cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the formation of tumors from the transplanted cell line. The invention also concerns a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of a receptor protein comprising (a) contacting cells from a stable breast cancer cell line with a drug candidate and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

One embodiment is a screening method comprising (a) contacting cells from a stable breast cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of HER2. Another embodiment the ability of the ADC candidate to block heregulin binding is evaluated. In another embodiment the ability of the ADC candidate to block ligand-stimulated tyrosine phosphorylation is evaluated.

performed using monolayer cultures on ice in a 24-well-plate format. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRGβ1$_{177-224}$ (25,000 cpm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC_{50} value may be calculated for the compound of interest.

Alternatively, or additionally, the ability of an ADC to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cell lines established from the transgenic animals herein may be incubated with a test ADC and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal antibody (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in US 5766863 is also available for determining ErbB receptor activation and blocking of that activity by the compound.

In one embodiment, one may screen for ADC which inhibit HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described below. For example, a cell line established from a HER2-transgenic animal may be plated in 24-well plates and the compound may be added to each well and incubated for 30 minutes at room temperature; then rHRGβ$_{1177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for about 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 µl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 µl) may be electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 µg/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r ~180,000 may be quantified by reflectance densitometry. An alternate method to evaluate inhibition of receptor phosphorylation is the KIRA (kinase receptor activation) assay (Sadick et al (1998) Jour. of Pharm. and Biomed. Anal. 1-9). Some of the well-established monoclonal antibodies against HER2 that are known to inhibit HRG stimulation of p180 tyrosine phosphorylation can be used as positive control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC_{50} for the compound of interest may be calculated.

One may also assess the growth inhibitory effects of a test ADC on cell lines derived from a HER2-transgenic animal (Schaefer et al (1997) Oncogene 15:1385-1394). According to this assay, the cells may be treated with a test compound at various concentrations for 4 days and stained with crystal violet or the redox dye Alamar Blue. Incubation with the compound may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4 on MDA-MB-175 cells (Schaefer et al., supra). In a further embodiment, exogenous HRG will not significantly reverse this inhibition.

To identify growth inhibitory ADC compounds that specifically target HER2, one may screen for ADC which inhibit the growth of HER2-overexpressing cancer cells derived from transgenic animals (US 5677171). According to this assay, HER2 overexpressing cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35mm dish) and the test compound is added at various concentrations. After six days, the number of cells, compared to untreated cells is counted using an electronic

Another embodiment is a screening method comprising (a) contacting cells from a stable breast cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

Another embodiment is a screening method comprising (a) administering an ADC drug candidate to a transgenic non-human mammal that overexpresses, e.g. in its mammary gland cells, a native human protein, e.g. HER2 or a fragment thereof, wherein such transgenic mammal has stably integrated into its genome a nucleic acid sequence encoding the native human protein or a fragment thereof having the biological activity of the native human protein, operably linked to transcriptional regulatory sequences directing its expression, and develops a tumor, e.g. a mammary tumor, not responding or poorly responding to antibody treatment, e.g. anti-HER2, or to a non-human mammal bearing a tumor transplanted from said transgenic non-human mammal; and (b) evaluating the effect of the ADC candidate on the target disease or disorder. Without limitations, the disease or disorder may be a HER2-overexpressing cancer, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic and bladder cancer. The cancer may be breast cancer which expressed HER2 in at least about 500,000 copies per cell, or at least about 2,000,000 copies per cell. ADC drug candidates may, for example, be evaluated for their ability to induce cell death and/or apoptosis, using assay methods well known in the art and described hereinafter.

In one embodiment, candidate ADC are screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. Administration may be oral, or by suitable injection, depending on the chemical nature of the compound being evaluated. In some cases, it may be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for compounds useful in treating various disorders associated with overexpression of certain tumor-associated antigen proteins or cell surface receptors, e.g. HER2-overexpression, the test compounds are added to the cell culture medium at an appropriate time, and the cellular response to the compound is evaluated over time using the appropriate biochemical and/or histological assays. In some cases, it may be appropriate to apply the compound of interest to the culture medium in conjunction with co-factors that would enhance the efficacy of the compound.

Thus, the present invention provides assays for identifying ADC which specifically target and bind the overexpressed HER2 protein, the presence of which is correlated with abnormal cellular function, and in the pathogenesis of cellular proliferation and/or differentiation of mammary gland that is causally related to the development of breast tumors.

To identify an ADC which blocks ligand activation of an ErbB (e.g. ErbB2) receptor, the ability of the compound to block ErbB ligand binding to cells expressing the ErbB (ErbB2) receptor (e.g. in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells isolated from the transgenic animal overexpressing HER2 and transfected to express another ErbB receptor (with which HER2 forms hetero-oligomer) may be incubated, i.e. culturing, with the ADC and then exposed to labeled ErbB ligand. The ability of the compound to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of heregulin (HRG) binding to breast tumor cell lines, overexpressing HER2 and established from the transgenic non-human mammals (e.g. mice) herein, by the candidate ADC may be

COULTER™ cell counter. Those ADC which inhibit cell growth by about 20-100% or about 50-100% may be selected as growth inhibitory compounds.

To select for ADC which induce cell death, loss of membrane integrity as indicated by, e.g., PI, trypan blue or 7AAD uptake may be assessed relative to control. The PI uptake assay uses cells isolated from the breast tumor tissue of a transgenic animal. According to this assay, the 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. The cells are seeded at a density of 3 x 106 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing various concentrations of the compound. The cells are incubated for a 3-day time period. Following each 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 may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing compounds.

In order to select for compounds which induce apoptosis, an annexin binding assay using cells established from the breast tumor tissue of the transgenic animal is performed. The cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 μg/ml of the antibody-drug conjugate (ADC). 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 above for the cell death assay. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing compounds.

IN VITRO CELL PROLIFERATION ASSAYS

Generally, the cytotoxic or cytostatic activity of an antibody-drug conjugate (ADC) is measured by: exposing mammalian cells having tumor-associated antigens or receptor proteins to the antibody of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based *in vitro* assays were used to measure viability, i.e. proliferation (IC_{50}), cytotoxicity (EC_{50}), and induction of apoptosis (caspase activation) of the ADC.

The *in vitro* potency of antibody-drug conjugates was measured by a cell proliferation assay (Figures 1-4). The CellTiter-Glo® Luminescent Cell Viability Assay is a commercially available (Promega Corp., Madison, WI), homogeneous assay method based on the recombinant expression of *Coleoptera* luciferase (US 5583024; US 5674713; US 5700670). This cell proliferation assay determines the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells (Crouch et al (1993) J. Immunol. Meth. 160:81-88; US 6602677). The CellTiter-Glo® Assay was conducted in 96 well format,

making it amenable to automated high-throughput screening (HTS) (Cree et al (1995) AntiCancer Drugs 6:398-404). The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent and mixing.

The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiter-Glo® Assay generates a "glow-type" luminescent signal, produced by the luciferase reaction, which has a half-life generally greater than five hours, depending on cell type and medium used. Viable cells are reflected in relative luminescence units (RLU). The substrate, Beetle Luciferin, is oxidatively decarboxylated by recombinant firefly luciferase with concomitant conversion of ATP to AMP and generation of photons. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch mode processing of multiple plates. This cell proliferation assay an be used with various multiwell formats, e.g. 96 or 384 well format. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is presented as relative light units (RLU), measured over time.

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow[Mg^{+2}]{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{light}$$

The anti-proliferative effects of three antibody-drug conjugates were measured by the cell proliferation, *in vitro* cell killing assay above against four different breast tumor cell lines (Figures 1-4). Figure 1 shows the potency measurements at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on SK-BR-3 (HER2 3+) breast tumor cells. Figure 2 shows the potency measurements of at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on BT-474 (HER2 3+) breast tumor cells. Figure 3 shows the potency measurements of at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on MCF7 (HER2 low) breast tumor cells. Figure 4 shows the potency measurements at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on MDA-MB-468 (HER2 negative) breast tumor cells.

IC_{50} values were established for SK-BR-3 and BT-474 which are known to overexpress HER2 receptor protein. A lot of conjugate trastuzumab-SPP-DM1 with 2.8 DM1 per trastuzumab (drug/Ab) gave a mean IC_{50} of 14.4 μg/ml with a range 9.1 to 22.3 μg/ml for 6 experiments against SK-BR-3 cells and a mean IC_{50} of 51.7 μg/ml with a range 28.7 to 63.1 μg/ml for 4 experiments against BT-474 cells. A lot of conjugate trastuzumab-SMCC-DM1 with 2.7 DM1 per trastuzumab (drug/Ab) gave a mean IC_{50} of 15.2 μg/ml with a range 12.6 to 18.8 μg/ml for 4 experiments against SK-BR-3 cells and a mean IC_{50} of 94.9 μg/ml with a range 75.2 to 114.6 μg/ml for 2 experiments against BT-474 cells. The conjugates were inactive against cells MCF7 and MDA-MB-468 which do not overexpress HER2.

AntiCD19-SMCC-DM1 showed potent cell killing *in vitro* with Raji cells (IC_{50} = <0.25 μg/ml) where the naked antibody and control ADC, trastuzumab-SMCC-DM1 showed no effect. AntiCD79a-SMCC-DM1 and antiCD79b-SMCC-DM1 showed potent cell killing in vitro with Ramos cells (IC_{50} = <0.25 μg/ml) where the naked antibody and control ADC, trastuzumab-SMCC-DM1 showed no effect.

Figure 17 shows an *in vitro*, cell proliferation assay (Example 5) with HT1080EphB2 (C8) cells treated with antiEphB2R 2H9 antibody-drug conjugates: 2H9-SPP-DM1 (IC_{50} 80 ng/ml), and 2H9-SMCC-DM1 (IC_{50} 50 ng/ml).

IN VIVO SERUM CLEARANCE AND STABILITY IN MICE

Serum clearance and stability of ADC were investigated in nude, naive (without tumors received by exogenous grafts) mice. Figure 5 shows the serum clearance in beige nude mice without tumors of trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, measuring conjugate and total antibody serum concentration at six time points over 7 days. A difference in the amount of total antibody and ADC indicates cleavage of the linker and separation of the antibody from its DM1 moiety. As illustrated by the higher conjugated antibody serum concentration at seven days, SMCC-linked ADC remained intact *in vivo* longer than did SPP-linked conjugates.

Figure 6 shows the stability over time in nude mice without tumors of the conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, and trastuzumab-SMCC-DM1, measuring serum concentration at six time points over 7 days. ADC with the SMCC linker were more stable *in vivo* than were conjugates linked by SPP or SPDP, although trastuzumab-SMCC-DM1 had approximately the same stability as the most hindered disulfide conjugate, trastuzumab-SPP-*DM4*.

The experiments shown in Figures 5 and 6 were performed in nude mice without tumors. However, SMCC-linked conjugates showed the same increased stability compared to SPP-linked conjugates in nude mice with tumors. As shown in Figure 7, approximately 72% of the initial trastuzumab-SMCC-DM1 remained as a conjugate 7 days after treatment, while only approximately 10% of the initial trastuzumab-SPP-DM1 remained as a conjugate 7 days after treatment, illustrating the improved stability of non-enzymatically cleavable trastuzumab-SMCC-DM1 conjugates in nude mice with tumors.

IN VIVO SERUM CLEARANCE AND STABILITY IN RATS

Figures 8 and 9 show the comparative stability and clearance profiles of a disulfide linker ADC (trastuzumab-SPP-DM1 and a non-disulfide linker ADC (trastuzumab-SMCC-DM1) in rats. The parameters of the study include:

Parameter	trastuzumab-SPP-DM1	trastuzumab-SMCC-DM1
Vd (ml/kg	41	41
Clearance (ml/day/kg)	52	15
T ½ alpha	0.09	0.15
T ½ beta	0.7	0.85
T ½ gamma (days)	2.6	5.5

The non-disulfide linker ADC, trastuzumab-SMCC-DM1 (Figure 9), showed better stability in rat serum than the disulfide linker ADC, trastuzumab-SPP-DM1 (Figure 8).

IN VIVO EFFICACY

The efficacy of the antibody-drug conjugates of the invention may be measured *in vivo* by implanting allografts or xenografts of cancer cells in rodents and treating the tumors with ADC. Variable results are to be expected depending on the cell line, the specificity of antibody binding of the ADC to receptors present on the cancer cells, dosing regimen, and other factors. The *in vivo* efficacy of anti-HER2 ADC was measured by a high expressing HER2 transgenic explant mouse model. An allograft was propagated from the Fo5 mmtv transgenic mouse which does not respond to, or responds poorly to, HERCEPTIN therapy. Subjects were treated once with ADC and monitored over 3-6 weeks to measure the time to tumor doubling, log cell kill, and tumor shrinkage. Follow up dose-response and multi-dose experiments were conducted.

Tumors arise readily in transgenic mice that express a mutationally activated form of *neu*, the rat homolog of HER2, but the HER2 that is overexpressed in breast cancers is not mutated and tumor formation is much less robust in transgenic mice that overexpress nonmutated HER2 (Webster et al (1994) Semin. Cancer Biol. 5:69-76).

To improve tumor formation with nonmutated HER2, transgenic mice were produced using a HER2 cDNA plasmid in which an upstream ATG was deleted in order to prevent initiation of translation at such upstream ATG codons, which would otherwise reduce the frequency of translation initiation from the downstream authentic initiation codon of HER2 (for example, see Child et al (1999) J. Biol. Chem. 274: 24335-24341). Additionally, a chimeric intron was added to the 5' end, which should also enhance the level of expression as reported earlier (Neuberger and Williams (1988) Nucleic Acids Res. 16: 6713; Buchman and Berg (1988) Mol. Cell. Biol. 8: 4395; Brinster et al (1988) Proc. Natl. Acad. Sci. USA 85:836). The chimeric intron was derived from a Promega vector, pCI-neo mammalian expression vector (bp 890-1022). The cDNA 3'-end is flanked by human growth hormone exons 4 and 5, and polyadenylation sequences. Moreover, FVB mice were used because this strain is more susceptible to tumor development. The promoter from MMTV-LTR was used to ensure tissue-specific HER2 expression in the mammary gland. Animals were fed the AIN 76A diet in order to increase susceptibility to tumor formation (Rao et al (1997) Breast Cancer Res. and Treatment 45:149-158).

Figures 10-13 show that the ADC have strong anti-tumor activity in the allograft of a HER2 positive tumor (Fo5) that originally arose in an MMTV-HER2 transgenic mouse. The antibody alone (e.g. trastuzumab) does not have significant anti-tumor activity in this model (Erickson et al US 6632979). As illustrated in

Figures 10 and 11, the growth of the tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth. Tumor growth was slowed most by treatment with trastuzumab-SMCC-DM1 and trastuzumab-SIAB-DM1 conjugates. As shown in Figures 10, 12 and 13, the trastuzumab-SMCC-DM1 conjugate slowed tumor growth more than conjugates with SPP linkers, i.e. more potency, whether measured as doubling time of tumors in nude mice or as Log Cell Kill corresponding to the doubling time measurements shown (Figure 13).

The *in vivo* efficacy of anti-CD22 ADC was measured with a mouse tumor xenograft model. Groups of eight SCID mice with 20 million Bjab-luc (luciferase expressing Bjab cells) xenograft tumor cells per mouse were dosed once at day 1 (except where noted) with an anti-CD22 antibody-drug conjugate, or a naked antibody (Example 8).

ADC or Ab	µg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
Tr-SMCC-DM1	200	4.2	3.2	3
7A2-SMCC-DM1	200	3.8	3.6	6
5B8-SMCC-DM1	200	3.8	3.6	10
RFB4-SMCC-DM1	200	3.2	4.25	18
RFB4-SMCC-DM1 (dosed 3X at 1, 7, 14 days)	405	10	2.75	55
7A2	-	4	-	3
5B8	-	4	-	3
RFB4	-	4	-	3

The time for tumor size to double was measured (MTD, mean tumor doubling time). The three naked anti-CD22 antibodies showed essentially no efficacy relative to a non-specific binding ADC (trastuzumab-SMCC-DM1). The corresponding conjugates all showed the effect of significantly retarding tumor growth. The effect of multiple dosing was established with RFB4-SMCC-DM1 where the MTD for singly dosed mice was 18 days whereas the MTD for mice dosed three times, at days 1, 7, and 14, was 55 days. Complete remission of tumor occurred in all 8 mice in the triple-dosed group. Other antiCD22-SMCC-DM1 conjugates, with antibodies 12F7, 9A8, 8C9, 8G10, 3F11, 10D2, 6C9, 14D1, and 11H10, showed shrinkage of initial tumor volume or retardation of tumor growth relative to control (trastuzumab-SMCC-DM1), after 7 days following a single dose (400 µg DM1/m2) in SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse. AntiCD22 conjugates, RFB4-SMCC-DM1, 5B8-SMCC-DM1, and 7A2-SMCC-DM1 were also effective in retardation of tumor growth relative to control (trastuzumab-SMCC-DM1), after 11 days following a single dose (200 µg DM1/m2) in SCID mice with 5 million Ramos RA1 xenograft tumor cells per mouse.

The conjugate RFB4-SMCC-DM1 was studied at three different drug loadings on groups of ten SCID mice with Bjab-luc xenografts (Example 8). The low (1.95) and medium (3.7) drug loaded conjugates each

showed the effect of significantly retarding tumor growth, with an MTD of about 15 days. The high loaded (6.75) conjugate did not show an effect significantly different than control conjugate GP120-SMCC-DM1, or naked antibody RFB4.

anti-CD22 ADC or Ab	μg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
RFB4	-	10	-	3
RFB4-SMCC-DM1 (low loaded)	144	5	1.95	15
RFB4-SMCC-DM1 (medium loaded)	273	5	3.7	15
RFB4-SMCC-DM1 (high loaded)	497	5	6.75	3
GP120-SMCC-DM1 (high loaded)	449	5	6.1	3

AntiCD19-SMCC-DM1 and antiCD22-SMCC-DM1 conjugates did not show *in vivo* activity in a Raji cell mouse tumor xenograft model. Other antiCD19 and antiCD22 conjugates may have *in vivo* activity against other cancer cell tumor models.

The *in vivo* efficacy of anti-CD79a (alpha) and anti-CD79b (beta) ADC was measured with a mouse tumor xenograft model. Groups of eight SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with samples in the table below and following Example 8.

ADC, Ab, or control	μg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
PBS (buffer control)	-	-	-	3.5
antiGP120	-	3.2	-	3.5
SN8 antiCD79b	-	3.1	-	4
17A7 antiCD79b	-	3.1	-	4
8H9 antiCD79a	-	4.0	-	3
antiGP120-SMCC-DM1	200	3.2	4.2	3.5
SN8 antiCD79b-SMCC-DM1	200	3.1	4.4	>7
17A7 antiCD79b-SMCC-DM1	200	3.1	4.4	>7
8H9 antiCD79a-SMCC-DM1	200	4.0	3.4	>7

Conjugates SN8 antiCD79b-SMCC-DM1, 17A7 antiCD79b-SMCC-DM1, and 8H9 antiCD79a-SMCC-DM1, all showed shrinkage of initial tumor volume (mean 160 mm3) after 7 days. In the groups of 8 mice, conjugate SN8 antiCD79b-SMCC-DM1 gave partial remission (PR) in 4 animals and complete remission (CR) in 2 animals. Conjugate 17A7 antiCD79b-SMCC-DM1 gave CR in 1 animal. Conjugate 8H9 antiCD79a-SMCC-DM1 gave PR in 2 animals and CR in 1 animal. Other antiCD79b-SMCC-DM1 conjugates, with antibodies 2F2, 5C3, 7H7, 8D11, 15B4, and 16C11, showed retardation of tumor growth or shrinkage of initial tumor volume, relative to control (trastuzumab-SMCC-DM1), after 8 days following a single dose (192 μg DM1/m2) in CB17 ICR SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse.

The dose response effect on mice administered with antiCD79b-SMCC-DM1 was measured. Groups of eight SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with samples in the table below (Example 8). The antiCD79b-SMCC-DM1 was dosed at levels of 0.5, 2.0, and 3.64 mg Ab/kg mouse.

ADC, Ab, or control	μg DM1/m2	Ab mg / kg mouse	MTD (days)
Vehicle control (PBS)	-		4
antiCD79b-SMCC-DM1	32	0.5	10
antiCD79b-SMCC-DM1	130	2.0	35
antiCD79b-SMCC-DM1	236	3.64	>70

The *in vivo* efficacy of antiTENB2 ADC was measured with a mouse tumor xenograft model. TENB2 is a tumor antigen that is shown to be almost exclusively expressed in the human prostate and overexpressed in human prostate tumors (Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84). PC3-TVA-919cv1:5 is a human prostate cancer cell line expressing high level of TENB2.

Athymic nude mice were subcutaneously injected with 5 million PC3-TVA-919 high expressor or medium expressor cells in a volume of 0.2 ml per mouse. Cells were suspended in HBSS. When mean tumor size reached 100-200 mm^3, the mice were randomly grouped into 8 groups of 8-10 mice each and give a single IV treatment of the samples below (Example 8).

ADC or control	µg DM1/m2	Ab mg / kg mouse	medium expressor MTD (days)	high expressor MTD (days)
PBS (vehicle control)	-	-	30	22
murine anti-ragweed-SPP-DM1 (negative control)	373	6.68	18	22
murine anti-ragweed-SMCC-DM1 (negative control)	373	9.34	14	18
murine antiTENB2:3146-SPP-DM1	373	7.34	58	35
murine antiTENB2:3146-SMCC-DM1	373	10.6	44	19
chimeric antiTENB2-SPP-DM1	373	8.7	57	43
chimeric antiTENB2-SMCC-DM1	373	7.63	43	22

Murine anti-TENB2-DM1 conjugates showed anti-tumor efficacy against PC3-TENB2 tumors, relative to negative control and vehicle control. Murine 10H1 antiNaPi3b-SMCC-DM1 conjugate showed no anti-tumor efficacy against PC3-NaPi3b tumors, relative to negative control and vehicle control. Other antibody variants of antiNaPi3b conjugates may have in vivo activity against PC3-NaPi3b tumors, or other cancer cell lines.

RODENT TOXICITY

Antibody-drug conjugates and an ADC-minus control, "Vehicle", were evaluated in an acute toxicity rat model. Toxicity of ADC was investigated by treatment of female Sprague-Dawley rats with the ADC and subsequent inspection and analysis of the effects on various organs. Based on gross observations (body weights), clinical pathology parameters (serum chemistry and hematology) and histopathology, the toxicity of ADC may be observed, characterized, and measured. It was found that at equivalent dose levels, trastuzumab-SMCC-DM1 was associated with less acute toxicity than trastuzumab-SPP-DM1.

A 5-day acute toxicity study in adolescent female rats (100-125 gms) was conducted by a single injection of trastuzumab-SMCC-DM1 (two doses: 1860 and 3260 µg DM1/m^2), a comparison disulfide ADC, trastuzumab-SPP-DM1 (two doses: 1860 and 3260 µg DM1/m^2), free DM1 maytansine (thiol) and a control Vehicle (day 0). Body weight was measured daily. Clinical chemistry, serum enzymes and hematology analysis was conducted on days 3 and 5; concluding with complete necropsy with histopathological assessment. Toxicity signals included the clinical observation of weight loss.

It is considered that weight loss, or weight change relative to animals dosed only with Vehicle in animals after dosing with ADC, is a gross and general indicator of systemic or localized toxicity. Figure 14 shows the changes in body weight (grams) over 5 days. Rats receiving the disulfide ADC, trastuzumab-SPP-DM1 showed a marked, dose-dependent toxicity, indicated by lethality at the higher dose and a decline in body

weight at the lower dose. In contrast, rats receiving trastuzumab-SMCC-DM1 gained weight, with the lower dosed rats showing no decline in the rate of weight gain, relative to the placebo Vehicle-dosed rats. Rats dosed at the higher level of trastuzumab-SMCC-DM1 also gained weight, comparable to the free DM1 cytotoxin.

Hepatotoxicity was measured by elevated liver enzymes, increased numbers of mitotic and apoptotic figures and hepatocyte necrosis. Hematolymphoid toxicity was observed by depletion of leukocytes, primarily granuloctyes (neutrophils), and/or platelets, and lymphoid organ involvement, i.e. atrophy or apoptotic activity. Toxicity was also noted by gastrointestinal tract lesions such as increased numbers of mitotic and apoptotic figures and degenerative entercolitis.

Enzymes indicative of liver injury that were studied include:

AST (aspartate aminotransferase)

--Localization: cytoplasmic; liver, heart, skeletal muscle, kidney

--Liver:Plasma ratio of 7000:1

--T1/2: 17 hrs

ALT (alanine aminotransferase)

--Localization: cytoplasmic; liver, kidney, heart, skeletal muscle

--Liver:Plasma ratio of 3000:1

--T1/2: 42 hrs; diurnal variation

GGT (g-glutamyl transferase)

--Localization: plasma membrane of cells with high secretory or absorptive capacity; liver, kidney, intestine

--Poor predictor of liver injury; commonly elevated in bile duct disorders

None of the three enzymes measured above are liver-specific. It was found that the ADC of the present invention caused a transient, mild elevation of liver enzymes ALT and AST, with transient reticulocytopenia (Figure 15). No significant effect on peripheral blood granulocytes or platelets was observed (Figure 16).

Figures 15 and 16 show that rats exposed to 22.3 mg/kg trastuzumab-SPP-DM1, (Group 2) demonstrated the most severe clinical toxicity in this five day acute toxicity study. These animals showed the most profound loss of body weight, elevations of liver function tests, leuko- and thrombocytopenia and morphologic evidence of toxicity against hematolymphoid tissues. The extent of toxicity was similar compared to that encountered in previous studies using a dose of 25mg/kg. In contrast, animals in Groups 3 and 4 given trastuzumab-SMCC-DM1 at 10 and 25 mg/kg, respectively, were indistinguishable from vehicle-treated animals based on clinical pathology and body weight data. Morphologically, these animals showed a mildly increased number of mitotic figures in the liver, however, peripheral lymphoid and hematopoietic tissues were within normal limits.

Group 5 animals, 50 mg/kg trastuzumab-SMCC-DM1, showed evidence of toxicity. However, with the exception of one liver function test (ALT), the severity of toxicity was less than in animals receiving 50% of the same drug dose as trastuzumab-SPP-DM1 (Group 2). At about the same dose, trastuzumab-SMCC-DM1 (Group 4, 22.3 mg/kg) showed about 25% of the AST level as trastuzumab-SPP-DM1 (Group 2, 25 mg/kg). By day 5 of this study, Group 5 animals showed increases in body weights (following a transitory loss during days 3 and 4), a decreasing serum bilirubin and a rising platelet count (Figure 15).

Animals exposed to free maytansinoid DM1 (Group 6) show the same pattern of toxicity as animals treated with trastuzumab conjugates. This dose of free DM1 corresponds to the amount of drug given as a 10 mg/kg trastuzumab-SPP-DM1 dose in previous studies. The severity of toxicity in animals of group 6 was less than that seen in animals of Group 2, but more than previously seen in animals treated with 10 mg/kg trastuzumab-SPP-DM1. Recovery appeared to be rather fast: The sections of spleen showed increased numbers of immature hematopoietic elements in animals treated with free maytansine; also, LFTs and clinical hematology parameters showed a distinct trend towards normalization in animals of Group 6 by day 5.

CYNOMOLGUS MONKEY TOXICITY/SAFETY

Toxicity and safety of ADC administered to Cynomolgus monkeys may be assessed. A toxicity/safety study of the antibody-drug conjugate, trastuzumab-SMCC-DM1, was conducted in Cynomolgus monkeys. Three groups of monkeys were studied to assess the toxicity of trastuzumab-SMCC-DM1 administered via intravenous injection at escalating doses, relative to control (Vehicle). Group 1 (4 subjects) received only Vehicle (PBS, pH 6.5, i.e. formulation minus ADC) at day 1 and day 22, followed by necropsy at day 36. Group 2 (4 subjects) received trastuzumab-SMCC-DM1 4900 μg/m2 at day 1 and at day 22. Group 3 (4 subjects) received trastuzumab-SMCC-DM1 7200 μg/m2 at day 22.

Hepatotoxicity was inferred by measurement of the elevated liver enzymes from the Rodent Toxicity study. Cynomolgus monkeys were dosed with Vehicle (Group 1) and trastuzumab-SMCC-DM1 (Group 2: 4900 $\mu g/m^2$; Group 3: 7200 $\mu g/m^2$. Liver enzyme AST, platelet counts, white blood cells, absolute neutrophil, red blood cells, reticulocytes, and a comparison of 2 IV dose regimens were measured for trastuzumab-SMCC-DM1 in Cynomolgus Monkeys (Example 10).

ADMINISTRATION OF ANTIBODY-DRUG CONJUGATE PHARMACEUTICAL FORMULATIONS

Therapeutic antibody-drug conjugates (ADC) 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, bolus, intratumor injection or epidural (Shire et al (2004) J. Pharm. Sciences 93(6):1390-1402). Pharmaceutical formulations of therapeutic antibody-drug conjugates (ADC) are typically prepared for parenteral administration with a pharmaceutically acceptable parenteral vehicle and in a unit dosage injectable form. An antibody-drug conjugate (ADC) having the desired degree of purity is optionally mixed with pharmaceutically acceptable diluents, carriers, excipients or stabilizers, in the form of a lyophilized formulation or an aqueous solution (Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.).

Acceptable parenteral vehicles, diluents, carriers, excipients, and stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose,

mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). For example, lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein by reference. An exemplary formulation of an ADC such as trastuzumab-SMCC-DM1 contains about 100 mg/ml of trehalose (2-(hydroxymethyl)-6-[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-tetrahydropyran-3,4,5-triol; $C_{12}H_{22}O_{11}$; CAS Number 99-20-7) and about 0.1% TWEEN™ 20 (polysorbate 20; dodecanoic acid 2-[2-[3,4-bis(2-hydroxyethoxy)tetrahydrofuran-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl ester; $C_{26}H_{50}O_{10}$; CAS Number 9005-64-5) at approximately pH 6.

Pharmaceutical formulations of a therapeutic antibody-drug conjugate (ADC) may contain certain amounts of unreacted drug moiety (D), antibody-linker intermediate (Ab-L), and/or drug-linker intermediate (D-L), as a consequence of incomplete purification and separation of excess reagents, impurities, and by-products, in the process of making the ADC; or time/temperature hydrolysis or degradation upon storage of the bulk ADC or formulated ADC composition. For example, a formulation of the ADC, trastuzumab-SMCC-DM1 may contain a detectable amount of free drug DM1. Alternatively, or *in addition to*, it may contain a detectable amount of drug-linker intermediate DM1-SMCC. Alternatively, or in addition to, it may contain a detectable amount of the antibody, trastuzumab. An exemplary formulation of trastuzumab-SMCC-DM1 may contain up to 10% molar equivalent of DM1-SMCC. Unexpectedly, it was determined by the *in vitro* cellular proliferation assay (Example 5), that DM1-SMCC (IC_{50} 0.05 μM) is about 20 times less potent in cell killing than free drug DM1 (IC_{50} 0.0045 μM) against SK-BR-3 and BT-474 breast cancer cells.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the ADC, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (US 3773919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, *degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™* (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

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

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared

by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Aqueous suspensions contain the active materials (ADC) in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, croscarmellose, povidone, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

The pharmaceutical compositions of ADC may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. Subcutaneous (bolus) administration may be effected with about 1.5 ml or less of total volume and a concentration of about 100 mg ADC per ml. For ADC that require frequent and chronic administration, the subcutaneous route may be employed, such as by pre-filled syringe or autoinjector device technology.

As a general proposition, the initial pharmaceutically effective amount of ADC administered per dose will be in the range of about 0.01-100 mg/kg, namely about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range of compound used being 0.3 to 15 mg/kg/day. For example, human patients may be initially dosed at about 1.5 mg ADC per kg patient body weight. This dose may be escalated to the maximally tolerated dose (MTD). The dosing schedule may be about every 3 weeks, but according to diagnosed condition or response, the schedule may be more or less frequent. The dose may be further adjusted during the course of treatment to be at or below MTD which can be safely administered for multiple cycles, such as about 4 or more.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

Although oral administration of protein therapeutics are generally disfavored due to poor bioavailability due to limited absorption, hydrolysis or denaturation in the gut, formulations of ADC suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the ADC.

The formulations may be packaged in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water, for injection immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Exemplary unit dosage formulations contain a daily dose or unit daily sub-dose, or an appropriate fraction thereof, of the active ingredient.

The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefore. Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered parenterally, orally or by any other desired route.

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, such as cancer and autoimmune conditions. Exemplary conditions or disorders include benign or malignant tumors; leukemia and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic disorders. Cancer susceptible to ADC treatment include those which are characterized by the overexpression of certain tumor associated antigens or cell surface receptors, e.g. HER2

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 similar to the clinical trials testing the efficacy of the anti-HER2 monoclonal antibody HERCEPTIN in patients with HER2 overexpressing metastatic breast cancers that had received extensive prior anti-cancer therapy as reported by Baselga et al. (1996) J. Clin. Oncol. 14:737-744. The clinical trial may be designed to evaluate the efficacy of an ADC in combination with known therapeutic regimens, such as radiation and/or chemotherapy involving known chemotherapeutic and/or cytotoxic agents (Pegram et al (1999) Oncogene 18:2241-2251).

Generally, the disease or disorder to be treated is cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, gastrointestinal stromal tumor (GIST), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

The cancer to be treated herein may be one characterized by excessive activation of an ErbB receptor, e.g. HER2. Such excessive activation may be attributable to overexpression or increased production of the ErbB receptor or an ErbB ligand. In one embodiment, a diagnostic or prognostic assay will be performed to determine whether the patient's cancer is characterized by excessive activation of an ErbB receptor. For example, ErbB gene amplification and/or overexpression of an ErbB receptor in the cancer may be determined. Various assays for determining such amplification/overexpression are available in the art and include the IHC, FISH and shed antigen assays described above. Alternatively, or additionally, levels of an ErbB ligand, such as TGF-alpha., in or associated with the tumor may be determined according to known procedures. Such assays may detect protein and/or nucleic acid encoding it in the sample to be tested. In one embodiment, ErbB ligand levels in the tumor may be determined using immunohistochemistry (IHC); see, for example, Scher et al. (1995) Clin. Cancer Research 1:545-550. Alternatively, or additionally, one may evaluate levels of ErbB ligand-encoding nucleic acid in the sample to be tested; e.g. via FISH, southern blotting, or PCR techniques. In one embodiment, ErbB2 overexpression may be analyzed by IHC, e.g. using the HERCEPTEST (Dako). Parrafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a ErbB2 protein staining intensity criteria as follows: Score 0, no staining is observed or membrane staining is observed in less than 10% of tumor cells; Score 1+, a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells, the cells are only stained in part of their membrane; Score 2+, a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells; Score 3+, a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells. Those tumors with 0 or 1+ scores for ErbB2 overexpression assessment may be characterized as not overexpressing ErbB2, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing ErbB2.

Alternatively, or additionally, FISH assays such as the INFORM™ (Ventana Co., Ariz.) or PATHVISION™ (Vysis, Ill.) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of ErbB2 overexpression in the tumor.

Moreover, ErbB receptor or ErbB ligand overexpression or amplification may be evaluated using an *in vivo* diagnostic assay, e.g. by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (e.g. a radioactive isotope) and externally scanning the patient for localization of the label.

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 the 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) 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, aromatase inhibitor, protein kinase inhibitor, lipid kinase inhibitor, anti-androgen, antisense oligonucleotide, ribozyme, gene therapy vaccine, anti-angiogenic 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 may also have a therapeutically effective amount of a chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, or a DNA binder.

Alternatively, or additionally, the second compound may be an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor. The second antibody may be monoclonal antibody 2C4 or humanized 2C4 "Omnitarg" (WO 01/00245). The second antibody may be conjugated with a cytotoxic or chemotherapeutic agent, e.g., a maytansinoid, an auristatin, a calicheamicin, or a 1,8 bis-naphthalimide moiety. For example, it may be desirable to further provide antibodies which bind to EGFR, ErbB2, ErbB3, ErbB4, or vascular endothelial factor (VEGF) in the one formulation or dosing regimen.

Other therapeutic regimens may be combined with the administration of an anticancer agent identified in accordance with this invention. 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 there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one embodiment, treatment with an ADC of the present invention 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, optionally along with treatment with an anti-ErbB2 antibody, such as trastuzumab. Chemotherapeutic agents include Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI), taxanes (such as paclitaxel and docetaxal) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers's 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 anticancer agent may be combined with an anti-hormonal compound; e.g., an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (EP 616812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is hormone independent cancer, the patient may previously have been subjected to anti-hormonal therapy and, after the cancer becomes hormone independent, the anti-ErbB2 antibody (and optionally other agents as described herein) may be

administered to the patient. It may be beneficial to also coadminister a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

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 may be 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.

Metabolites include the products of *in vivo* cleavage of the ADC where cleavage of any bond occurs that links the drug moiety to the antibody. Metabolic cleavage may thus result in the naked antibody, or an antibody fragment. The antibody metabolite may be linked to a part, or all, of the linker. Metabolic cleavage may also result in the production a drug moiety or part thereof. The drug moiety metabolite may be linked to a part, or all, of the linker.

ARTICLES OF MANUFACTURE

In another embodiment, an article of manufacture, or "kit", containing ADC and 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, or blister pack. 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. In one embodiment, the label or package inserts indicates that the composition comprising the antibody which binds ErbB2 can be used to treat cancer which expresses an ErbB receptor selected from the group consisting of epidermal growth factor receptor (EGFR), ErbB2, ErbB3 and ErbB4. In addition, the label or package insert may indicate that the patient to be treated is one having cancer characterized by excessive activation of an ErbB receptor selected from EGFR, ErbB2, ErbB3 or ErbB4. For example, the cancer may be one which overexpresses one of these receptors and/or which overexpresses an ErbB ligand (such as TGF-α). The label or package insert may also indicate that the composition can be used to treat cancer, wherein the cancer is not characterized by overexpression of the ErbB2 receptor. In other embodiments, the package insert may indicate that the ADC composition can be used also to treat hormone independent cancer, prostate cancer, colon cancer or colorectal cancer.

The article of manufacture may comprise (a) a first container with a compound contained therein, wherein the compound comprises an ADC of the present invention in which the antibody of the ADC is a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second container with a compound contained therein, wherein the compound comprises a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, or a conjugate of this second antibody with a maytansinoid. The article of manufacture in this embodiment may further comprise a package insert indicating that the first and second compounds can be used to treat 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.

EXAMPLES

Example 1 - Production, Characterization and Humanization of Anti-ErbB2 Monoclonal Antibody 4D5

The murine monoclonal antibody 4D5 which specifically binds the extracellular domain of ErbB2 was produced as described in Fendly et al (1990) Cancer Research 50:1550-1558. Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al (1987) Proc. Natl. Acad. Sci. (USA) 84:7158-7163 were harvested with phosphate buffered saline (PBS) containing 25mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P-labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13.

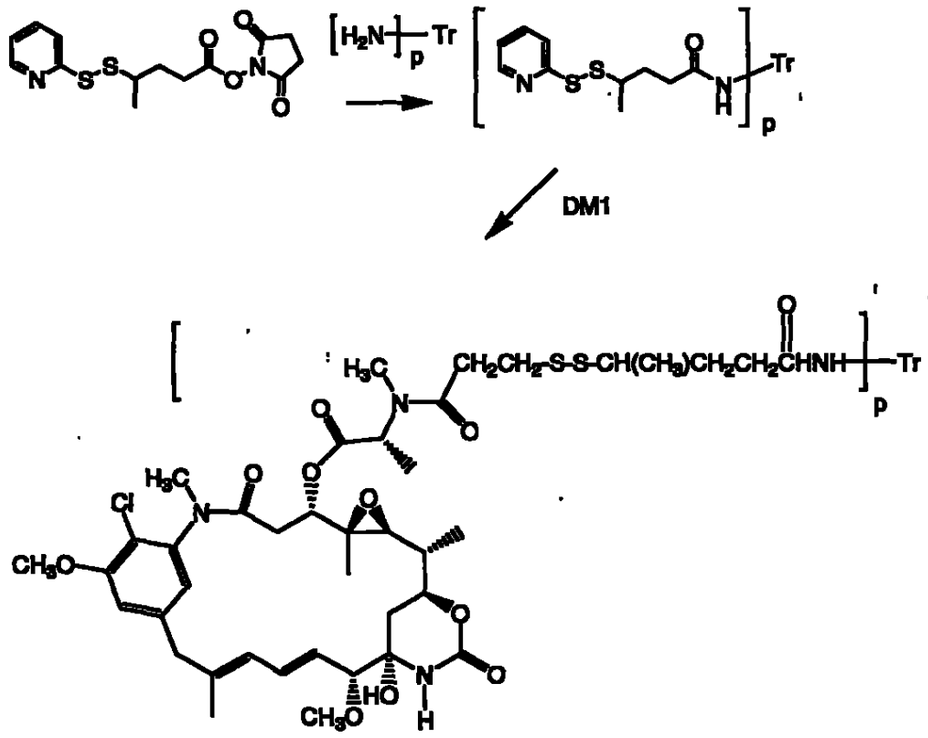
This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

The murine monoclonal antibody 4D5 was humanized, using a "gene conversion mutagenesis" strategy, as described in US 5821337, the entire disclosure of which is hereby expressly incorporated by reference. The humanized monoclonal antibody 4D5 used in the following experiments is designated huMAb4D5-8. This antibody is of IgG1 isotype.

Example 2 - Purification of Trastuzumab

One vial containing 440 mg HERCEPTIN® (huMAb4D5-8, rhuMAb HER2, US 5821337) antibody) was dissolved in 50 mL MES buffer (25 mM MES, 50 mM NaCl, pH 5.6) and loaded on a cation exchange column (Sepharose S, 15 cm x 1.7 cm) that had been equilibrated in the same buffer. The column was then washed with the same buffer (5 column volumes). Trastuzumab was eluted by raising the NaCl concentration of the buffer to 200 mM. Fractions containing the antibody were pooled, diluted to 10 mg/mL, and dialyzed into a buffer containing 50 mM potassium phosphate, 50 mM NaCl, 2 mM EDTA, pH 6.5.

Example 3 - Preparation of Antibody-Drug Conjugates: Trastuzumab-SPP-DM1:



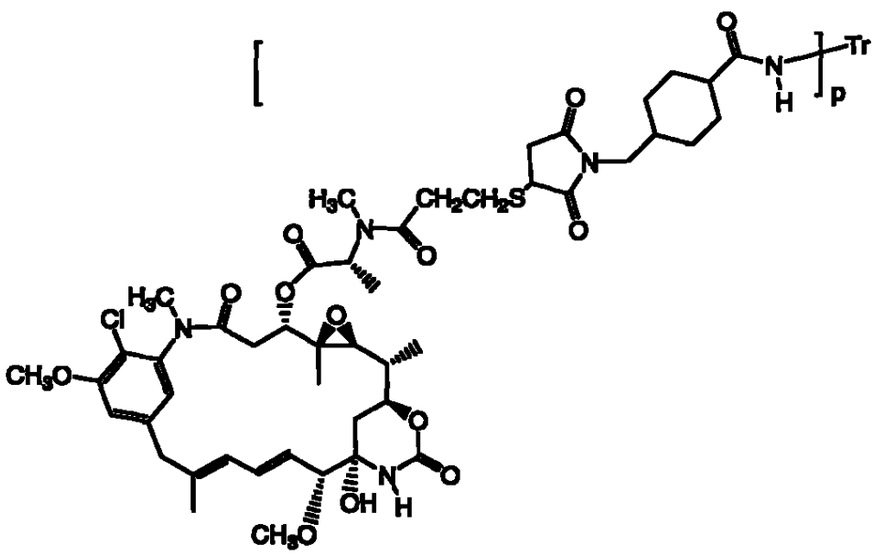
Purified trastuzumab was derivatized with N-succinimidyl-4-(2-pyridylthio)pentanoate to introduce dithiopyridyl groups. Trastuzumab (376.0 mg, 8 mg/mL) in 44.7 mL of 50 mM potassium phosphate buffer (pH 6.5) containing NaCl (50 mM) and EDTA (1 mM) was treated with SPP (5.3 molar equivalents in 2.3 mL

ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture was gel filtered through a *Sephadex G25* column equilibrated with 35 mM sodium citrate, 154 mM NaCl, 2 mM EDTA. Antibody containing fractions were pooled and assayed. The degree of modification of the antibody was determined as described above. Recovery of the modified antibody (trastuzumab-SPP-Py) was 337 mg (89.7%) with 4.5 releasable 2-thiopyridine groups linked per antibody (p').

Trastuzumab-SPP-Py (337.0 mg, 9.5 μmols of releasable 2-thiopyridine groups) was diluted with the above 35 mM sodium citrate buffer, pH 6.5, to a final concentration of 2.5 mg/mL. DM1 ($N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine), the structure of which is shown in Figure 1, (1.7 equivalents, 16.1 μmols) in 3.0 mM dimethylacetamide (DMA, 3% v/v in the final reaction mixture) was then added to the antibody solution. The reaction proceeded at ambient temperature under argon for 20 hours.

The reaction was loaded on a Sephacryl S300 gel filtration column (5.0 cm x 90.0 cm, 1.77 L) equilibrated with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate was 5.0 mL/min and 65 fractions (20.0 mL each) were collected. A major peak centered around fraction No. 47 (Figure 3). The major peak comprises monomeric trastuzumab-SPP-DM1. Fractions 44-51 were pooled and assayed. The number of DM1 drug molecules linked per antibody molecule (p') was determined by measuring the absorbance at 252 nm and 280 nm, and found to be 3.7 drug molecules per antibody molecule.

Example 4 - Preparation of Antibody-Drug Conjugates: Trastuzumab-SMCC-DM1



Purified trastuzumab was derivatized with (Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate, SMCC, Pierce Biotechnology, Inc) to introduce the SMCC linker. Trastuzumab was purified from HERCEPTIN® as in Example 2 and buffer-exchange treated at 20 mg/mL in 50 mM potassium phosphate/ 50 mM sodium chloride/ 2 mM EDTA, pH 6.5 with 7.5 to 10 molar equivalents of SMCC (20 mM in DMSO or DMA (dimethylacetamide), 6.7 mg/mL). After stirring for 2 to 4 hours under argon at ambient temperature, the reaction mixture was filtered through a Sephadex G25 column equilibrated with 50mM potassium phosphate/ 50 mM sodium chloride/ 2 mM EDTA, pH 6.5. Alternatively, the reaction mixture was gel filtered with 30 mM

citrate and 150 mM sodium chloride at pH 6. Antibody containing fractions were pooled and assayed. Recovery of trastuzumab-SMCC was 88%.

The drug-linker intermediate, trastuzumab-SMCC from above, was diluted with 50 mM potassium phosphate/50 mM sodium chloride/2 mM EDTA, pH 6.5, to a final concentration of 10 mg/ml, and reacted with a 10 mM solution of DM1 (1.7 equivalents assuming 5 SMCC/trastuzumab, 7.37 mg/ml) in dimethylacetamide. The reaction was stirred at ambient temperature under argon for 4 to about 16 hours. The conjugation reaction mixture was filtered through a Sephadex G25 gel filtration column (1.5 x 4.9 cm) with 1 x PBS at pH 6.5. Alternatively, the reaction mixture was gel filtered with 10 mM succinate and 150 mM sodium chloride at pH 5. The DM1/trastuzumab ratio (p) was 3.1, as measured by the absorbance at 252 nm and at 280 nm. The drug to antibody ratio (p) may also be measured by mass spectrometry. Conjugation may also be monitored by SDS polyacrylamide gel electrophoresis. Aggregation may be assessed by laser light scattering analysis.

Antibody-SMCC-DM1 Conjugates:

Following this protocol, other antibody-drug conjugates with the SMCC linker and DM1 drug moiety were prepared, including:

Antibody-drug conjugate (ADC)	antibody (Ab)	antigen	average drug loading (DM1/Ab)
10H-SMCC-DM1	10H1 (murine) anti-NaPi3b	NaPi3b	5.0
2H9-SMCC-DM1	2H9 Mab	anti-EphB2R	4.0
ragweed-SMCC-DM1	ragweed		2.7
antiTENB2-SMCC-DM1	anti-TENB2-3416#2 Mouse MAb	TENB2	2.5
anti-TENB2 20D1 Fc Chimera-SMCC-DM1	Chimeric Human Mouse anti-TENB2	TENB2	3.32
antiCD19-SMCC-DM1	antiCD19 (mIgG1 isotype)	CD19	5.05
2H7-SMCC-DM1	antiCD20 (mIgG2a isotype)	CD20	2.44, 3.84 (two lots)
CD20 LC-SMCC-DM1	antiCD20 (Rituxan®)	CD20	
HB5-SMCC-DM1	antiCD21 (mIgG2a isotype)	CD21	4.05
RFB4-SMCC-DM1	antiCD22	CD22	1.95-6.75 (multiple lots)
Cy34.1.2-SMCC-DM1	antiCD22	CD22	2.70
12F7.1.5-SMCC-DM1	antiCD22	CD22	5.90
9A8.1.1-SMCC-DM1	antiCD22	CD22	5.00

8C9.1.2-SMCC-DM1	antiCD22	CD22	2.90
14B3.3.1-SMCC-DM1	antiCD22	CD22	5.20
8G10.4.2-SMCC-DM1	antiCD22	CD22	3.60
2B4.1.4-SMCC-DM1	antiCD22	CD22	5.20
7A2.4.1-SMCC-DM1	antiCD22	CD22	3.60
4H3.2.2-SMCC-DM1	antiCD22	CD22	3.60
5E8.1.8-SMCC-DM1	antiCD22	CD22	3.60
3F11.2.1-SMCC-DM1	antiCD22	CD22	4.50
6C9.1.3-SMCC-DM1	antiCD22	CD22	3.70
10D2.4.3-SMCC-DM1	antiCD22	CD22	3.65
10D6.8.1-SMCC-DM1	antiCD72	CD72	2.90
ZL7-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
5C3-SMCC-DM1	antiCD79a (alpha)	CD79a	1.85
6G1-SMCC-DM1	antiCD79a (alpha)	CD79a	3.50
7H7-SMCC-DM1	antiCD79a (alpha)	CD79a	3.25
8D11-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
8H9-SMCC-DM1	antiCD79a (alpha)	CD79a	3.40
11E5-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
15E4-SMCC-DM1	antiCD79a (alpha)	CD79a	3.25
16C11-SMCC-DM1	antiCD79a (alpha)	CD79a	2.50
2F2-SMCC-DM1	antiCD79b (beta)	CD79b	3.20
17A7-SMCC-DM1	antiCD79b (beta)	CD79b	4.40
SN8-SMCC-DM1	antiCD79b (beta)	CD79b	4.40, 0.80 (two lots)
1F9-SMCC-DM1	antiFcRH1	FcRH1	4.20
7A2-SMCC-DM1	antiFcRH2	FcRH2	4.20, 3.95 (two lots)
7G7-SMCC-DM1	antiFcRH2	FcRH2	3.80
cl1D6-SMCC-DM1	antiFcRH2	FcRH2	2.20
51505.111-SMCC-DM1	antiCXCR5	CXCR5	6.10

12C7 Fc Chimera-SMCC-DM1	antiBrevican	brevican	4.60
H2:PSCA hIog-SMCC-DM1	antiPSCA hIog	PSCA	3
H6:xPSCAhIog-SMCC-DM1	antiPSCA hIog	PSCA	2.6
xFcRH5:3909 7D11.1.1-SMCC-DM1	antiIRTA2	IRTA2	2.6
xFCRH5.7D11-SMCC-DM1	antiIRTA2	IRTA2	4.4
11D10 LC-SMCC-DM1	Mouse anti-CA 0772P MAb	CA 0772P MUC16	
xNCA LC-SMCC-DM1	Mouse anti-CEACAM6 MAb	CEACAM6 NCA	

Antibody-BMPEO-DM1 Conjugates:

For cysteine conjugation, the antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, in the presence of EDTA. After incubating for about one hour at 37 °C, the pH is adjusted to about 7 with 100 mM potassium phosphate. The reduced antibody is modified by the bis-maleimido reagent BM(PEO)4 (Pierce Chemical), leaving an unreacted maleimido group on the surface of the antibody. This may be accomplished by dissolving BM(PEO)4 in a 50% ethanol/water mixture to a concentration of 10 mM and adding a tenfold molar excess to a solution containing antibody in phosphate buffered saline at a concentration of approximately 1.6 mg/ml (10 μM) and allowing it to react for 1 hour to form Ab-BMPEO. Excess BM(PEO)4 is 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 is dissolved in dimethyl acetamide (DMA) and added to the Ab-BMPEO intermediate. Dimethyl formamide (DMF) may also be employed to dissolve the drug moiety reagent. The reaction mixture is allowed to react overnight before gel filtration or dialysis into PBS to remove unreacted DM1. Gel filtration on S200 columns in PBS was used to remove high molecular weight aggregates and furnish purified Ab-BMPEO-DM1.

Following this protocol, other antibody-drug conjugates with the BMPEO linker and DM1 drug moiety were prepared, including:

Antibody-drug conjugate (ADC)	antibody (Ab)	antigen	average drug loading (DM1/Ab)
trastuzumab-BM(PEO)-DM1	trastuzumab (Herceptin®)	HER2	2.94
CD120-BMPEO4-DM1	Mouse anti-GP120 MAb	GP120	2
RFB4-BMPEO4-DM1	antiCD22	CD22	3.7 and 4.25 (two lots)

Example 5 - In vitro cell proliferation assay

Efficacy of ADC were measured by a cell proliferation assay employing the following protocol (Promega Corp. Technical Bulletin TB288; Mendoza et al (2002) Cancer Res. 62:5485-5488):

1. An aliquot of 100 µl of cell culture containing about 10^4 cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.
2. Control wells were prepared containing medium and without cells.
3. ADC was added to the experimental wells and incubated for 3-5 days.
4. The plates were equilibrated to room temperature for approximately 30 minutes.
5. A volume of CellTiter-Glo Reagent equal to the volume of cell culture medium present in each well was added.
6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.
7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.
8. Luminescence was recorded and reported in graphs as RLU = relative luminescence units.

Example 6 - Serum clearance and stability in mice

A beige mutation, nude, naive (without tumors) mice in six Groups of four animals were studied. At Day 0, each mouse received a single 2 mg/kg dose of ADC in 200 µl aqueous carrier, except the Vehicle Group which received only carrier. Blood was collected by cardiac puncture under anesthesia at each time point (5 minutes, 1 hour, 6 hours, 24 hours, 72 hours, and 168 hours) post-dose. The serum was isolated and antibody and ADC were measured.

Group 1: Vehicle (PBS, pH 6.5, no ADC)
Group 2: Trastuzumab-SMCC-DM1
Group 3: Trastuzumab-SPP-DM1
Group 4: Trastuzumab-SPDP-DM1
Group 5: Trastuzumab-SPP-DM3
Group 5: Trastuzumab-SPP-DM4

Example 7 - Serum stability in rats

Six dose groups with 6 Sprague-Dawley rats (100-125 gms each) per dose group were studied. At Day 0, Animals were administered a single IV dose of Vehicle, 10 mg/kg trastuzumab-SPP-DM1, or 10 mg/kg trastuzumab-SMCC-DM1, at a dose volume of 10 ml/kg via the lateral tail vein. Approximately 300 µl whole blood was collected at each timepoint: 0 (predose), 10, and 30 minutes; 1, 2, 4, 8, 24 and 36 hours; and 2, 3, 4, 7, 14, 21, 28 days post dose.

Example 8 - Tumor volume in vivo efficacy

High expressing HER2 transgenic explant mice:

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but FVB female mice are preferred because of their higher susceptibility to tumor formation. FVB males were used for mating and vasectomized CD.1 studs were used to stimulate pseudopregnancy. Vasectomized mice can be obtained from any commercial supplier.

Founders were bred with either FVB mice or with 129/BL6 x FVB p53 heterozygous mice. The mice with heterozygosity at p53 allele were used to potentially increase tumor formation. However, this has proven unnecessary. Therefore, some F1 tumors are of mixed strain. Founder tumors are FVB only. Six founders were obtained with some developing tumors without having litters.

Animals having tumors (allograft propagated from Fo5 mmtv transgenic mice) were treated with a single injection of a trastuzumab-DM1 maytansinoid conjugate (10 mg/kg dose) and tumor volume was assessed for over 20 days after injection.

Bjab-luc xenograft SCID mice:

Groups of eight to ten SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with an antibody-drug conjugate, or a naked antibody. A group of eight mice were treated each with anti-CD22 ADC, naked anti-CD22 antibodies, and control. Control ADC (trastuzumab-SMCC-DM1; loading: DM1/trastuzumab =3.2) is not a specific binder and was administered at 200 μg DM1/m2, 4.2 trastuzumab/kg mouse, and resulted in a mean tumor doubling time of about 3 days. Anti-CD22 conjugates 7A2-SMCC-DM1 (loading: DM1/Ab = 3.6), 5E8-SMCC- DM1 (loading: DM1/Ab = 3.6), and RFB4-SMCC-DM1 (loading: DM1/Ab = 4.3). Naked antibodies, 7A2, 5E8, and RFB4 were dosed at 4 mg/kg mouse.

Example 9 - Rat toxicity

The acute toxicity profile of trastuzumab-SPP-DM1 (disulfide linker) compared with that of free DM1 and trastuzumab-SMCC-DM1 (non-disulfide linker) was evaluated). Animals were injected on day 1, complete chemistry and hematology profiles were obtained at baseline, day 3 and day 5 and a complete necropsy was performed on day 5. Routine histology was performed on three random animals for each group for the following tissues: sternum, liver, kidney, thymus, spleen, large and small intestine. The experimental groups were as follows:

Group 1: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0)
Group 2: Trastuzumab-SPP-DM1, 22.3mg/kg
Group 3: Trastuzumab-SMCC-DM1, 10mg/kg
Group 4: Trastuzumab-SMCC-DM1, 25mg/kg
Group 5: Trastuzumab-SMCC-DM1, 50mg/kg
Group 6: Free DM1, 160μg/kg

Example 10 - Cynomolgus Monkey Toxicity/Safety

Three groups of four (2 male, 2 female) naive *Macaca fascicularis* (cynomolgus monkey) were studied.

Group 1: (4 animals) received only Vehicle (PBS, pH 6.5, i.e. formulation minus ADC) at day 1 and day 22, followed by necropsy at day 36.
Group 2: (4 animals) received trastuzumab-SMCC-DM1 4900 μg/m2 at day 1 and at day 22.
Group 3: (4 animals) received trastuzumab-SMCC-DM1 7200 μg/m2 at day 22.

Dosing is expressed in surface area of an animal so as to be relevant to other species, i.e. dosage at μg/m2 is independent of species and thus comparable between species. Formulations of trastuzumab-SMCC-

DM1 for Group 2 and Group 3 studies contained PBS, 5.4mM sodium phosphate, 4.2 mM potassium phosphate, 140 mM sodium chloride, pH 6.5.

Blood was collected for hematology analysis prior to first dosing (acclimation period) and at days 3, 7, 11, and 14 after the first dose (Groups 1 and 2) and at days 3, 7, 11, 14, and 21 after the second dose (Groups 1, 2 and 3). Erythrocyte (RBC) and platelet (PLT) counts were measured by the light scattering method. Leukocyte (WBC) count was measured by the peroxidase/basophil method. Reticulocyte count was measured by the light scattering method with cationic dye. Cell counts were measured on an Advia 120 apparatus. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in U/L by UV/NADH; IFCC methodology on an Olympus AU400 apparatus, and using Total Ab ELISA - ECD/GxhuFc-HRP. Conj. Ab ELISA - xDM1/ECD-Bio/SA-HRP tests.

All patents, patent applications, and references cited throughout the specification are expressly incorporated by reference.

We claim:

1. An antibody-drug conjugate compound comprising an antibody covalently attached by a linker to one or more maytansinoid drug moieties, the compound having Formula I:

$$Ab–(L–D)_p \qquad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

provided that the antibody is not TA.1;

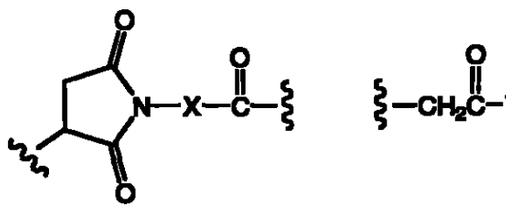
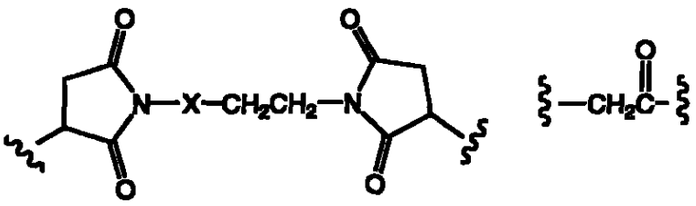
L is a non-disulfide linker;

D is a maytansinoid drug moiety; and

p is 1 to 8.

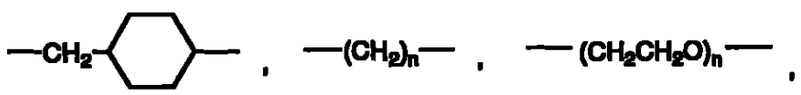
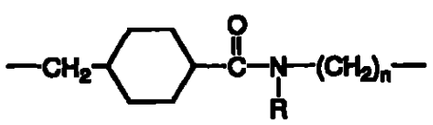
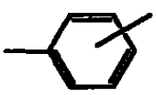
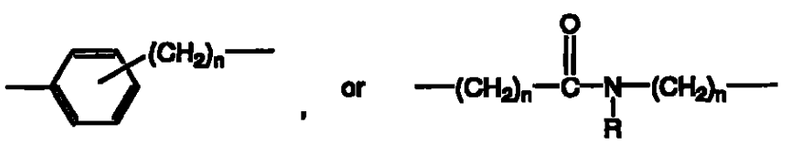
2. The antibody-drug conjugate compound of claim 1 wherein Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36).

3. The antibody-drug conjugate compound of claim 1 or claim 2 wherein L is a linker selected from the structures:

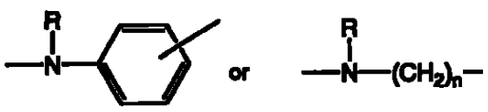



where the wavy lines indicate the covalent attachments to Ab and D;

X is:

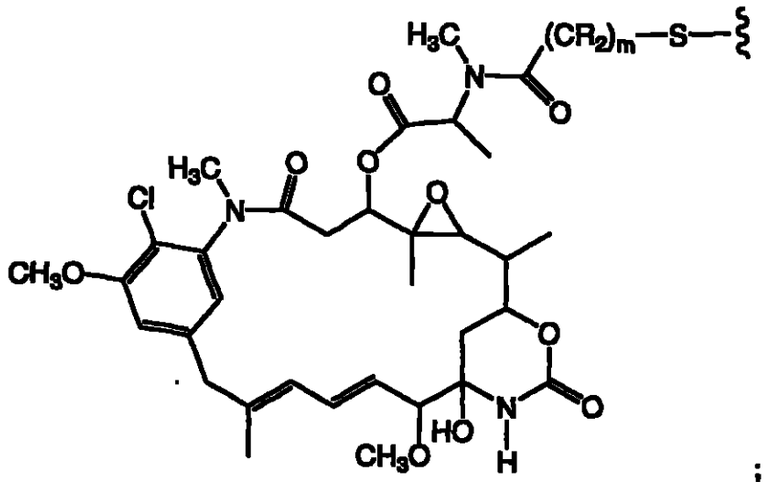





Y is:



R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

4. The antibody-drug conjugate compound of any of the preceding claims wherein D is selected from the structure:



;

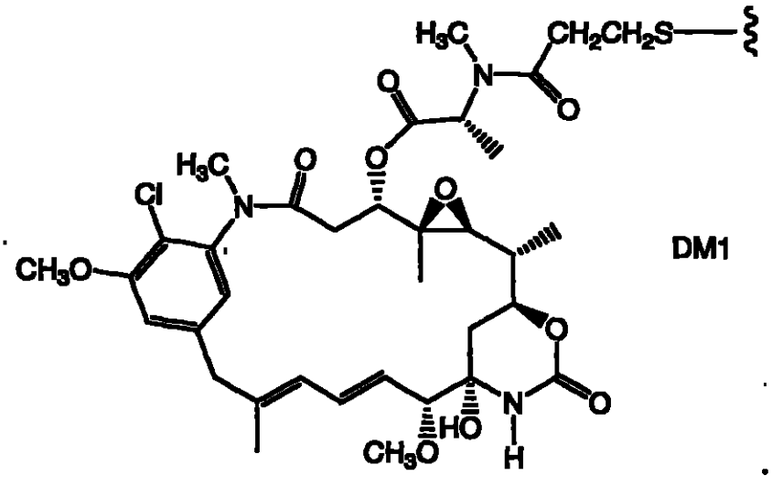
where the wavy line indicates the covalent attachment to L,

R is independently H or C_1–C_6 alkyl, and

m is 1, 2, or 3.

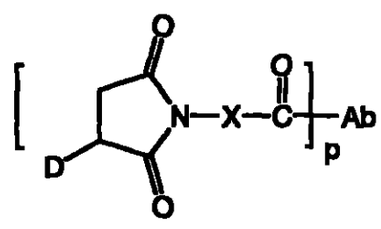
5. The antibody-drug conjugate compound of claim 4 wherein m is 2, and R is H.

6. The antibody-drug conjugate compound of any of the preceding claims wherein the maytansinoid drug moiety is DM1, having the structure:

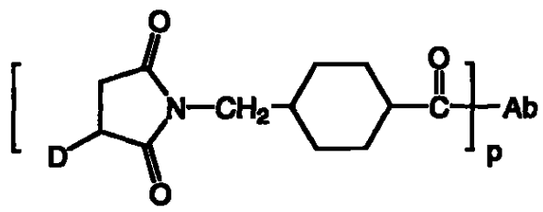


DM1

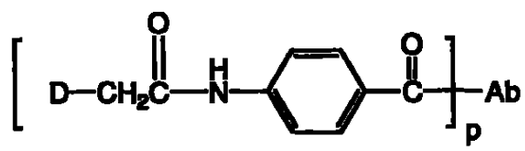
7. The antibody-drug conjugate compound of claim 3 having the structure:



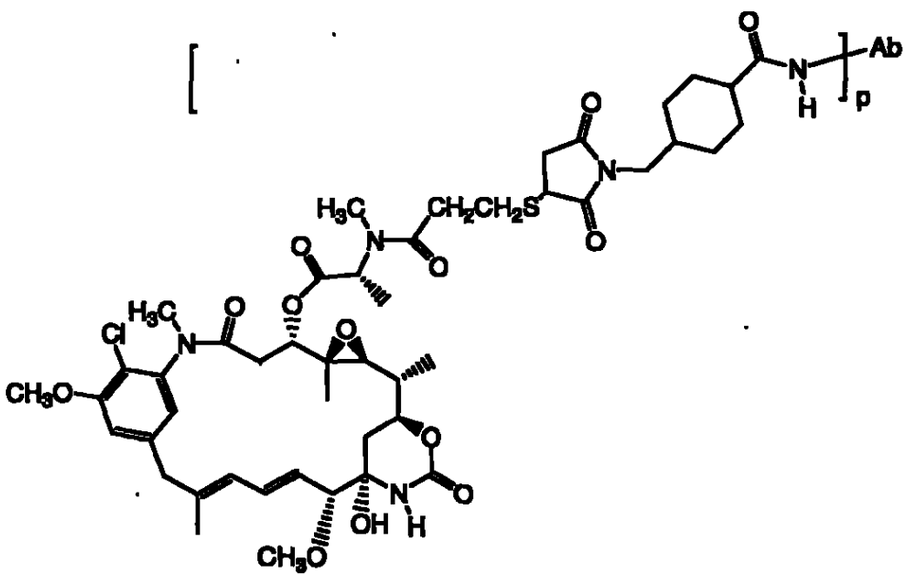
8. The antibody-drug conjugate compound of claim 7 having the structure:



9. The antibody-drug conjugate compound of claim 3 having the structure:

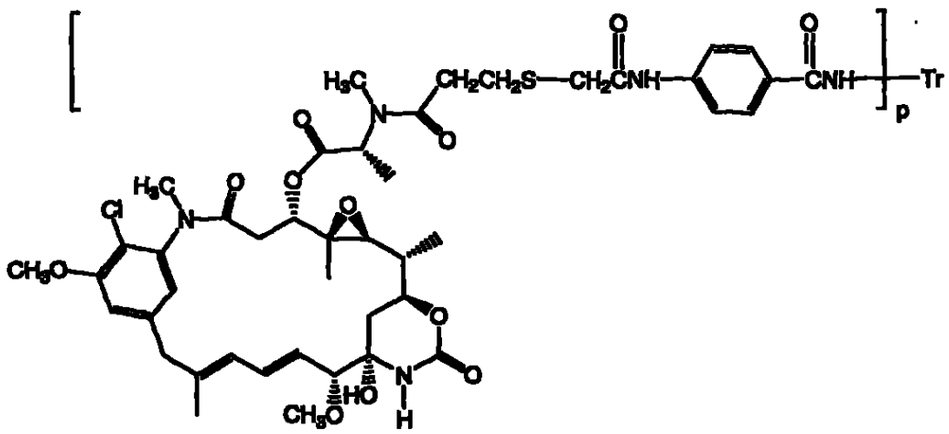


10. The antibody-drug conjugate compound of claim 1 having the structure:



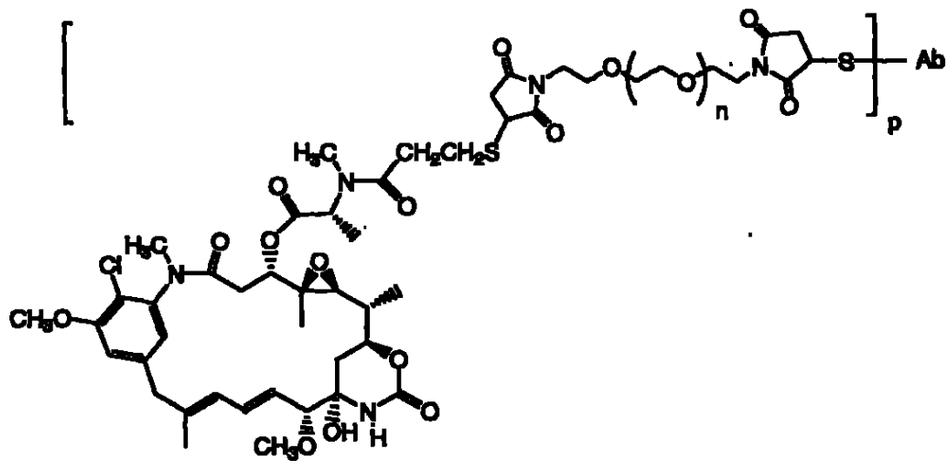
11. The antibody-drug conjugate of claim 10 wherein Ab is trastuzumab, and p is 1, 2, 3, or 4.

12. The antibody-drug conjugate compound of claim 1 having the structure:



where Tr is trastuzumab, and p is 1, 2, 3, or 4.

13. The antibody-drug conjugate compound of claim 1 having the structure:



wherein n is 0, 1, or 2; and p is 1, 2, 3, or 4.

14. The antibody-drug conjugate compound of claim 13 wherein Ab is trastuzumab.

15. The antibody-drug conjugate compound of claim 1 wherein p is 1, 2, 3, or 4.

16. The antibody-drug conjugate compound of claim 1 wherein the antibody binds to a receptor encoded by an ErbB gene.

17. The antibody-drug conjugate compound of claim 16 wherein the receptor is selected from EGFR, HER2, HER3 and HER4.

18. The antibody-drug conjugate compound of claim 17 wherein the antibody specifically binds to a HER2 receptor.

19. The antibody-drug conjugate compound of claim 1 which specifically binds to the extracellular domain of the HER2 receptor and inhibits growth of tumor cells which overexpress HER2 receptor.

20. The antibody-drug conjugate compound of any of the preceding claims wherein the antibody is selected from a monoclonal antibody, an antibody fragment, a chimeric antibody, and a humanized antibody.

21. The antibody-drug conjugate compound of claim 1 wherein the antibody is a humanized antibody selected from huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (trastuzumab).

22. The antibody-drug conjugate compound of claim 21 wherein the antibody is huMAb4D5-8 (trastuzumab).

23. The antibody-drug conjugate of claim 20 wherein the antibody is a Fab fragment.

24. The antibody-drug conjugate compound of claim 1 wherein the antibody is attached to the linker through a cysteine thiol of the antibody.

25. The antibody-drug conjugate compound of claim 24 wherein p is 1, 2, 3, or 4.

26. A pharmaceutical composition comprising the antibody-drug conjugate compound of claim 1 or claim 2, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

27. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of a chemotherapeutic agent selected from Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozole, Imatinib mesylate, PTK787/ZK 222584, Oxaliplatin, 5-FU, Leucovorin, Rapamycin, Lapatinib, Lonafarnib, Sorafenib, and Gefitinib.

28. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of a anti-angiogenic agent.

29. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of bevacizumab.

30. A method of inhibiting cellular proliferation comprising treating mammalian cells in a cell culture medium with an antibody-drug conjugate compound of claim 1 or claim 2, whereby proliferation of the cells is inhibited.

31. The method of claim 30 wherein the mammalian cells have HER2 receptor proteins to the antibody-drug conjugate compound.

32. The method of claim 31 wherein the mammalian cells are breast tumor cells.

33. The method of claim 30 wherein the antibody-drug conjugate compound is more cytotoxic than a maytansinoid compound comprising the maytansinoid moiety of the antibody-drug conjugate compound.

34. The method of claim 30 wherein the antibody-drug conjugate induces apoptosis.

35. A method of treating cancer comprising administering to a patient a formulation of an antibody-drug conjugate compound of claim 1 or claim 2, and a pharmaceutically acceptable diluent, carrier or excipient.

36. The method of claim 35 wherein the cancer is selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

37. The method of claim 36 wherein the cancer is breast cancer which overexpresses ErbB2 at a 2+ level or more.

38. The method of claim 35 wherein the amount of antibody-drug conjugate compound administered to the patient is in the range of about 0.1 to about 10 mg/kg of patient weight per dose.

39. The method of claim 35 wherein the antibody-drug conjugate is administered at about three week intervals.

40. The method of claim 35 wherein the antibody-drug conjugate is administered by infusion.

41. The method of claim 35 wherein the antibody-drug conjugate is formulated with a pharmaceutically acceptable parenteral vehicle.

42. The method of claim 41 wherein the antibody-drug conjugate is formulated in a unit dosage injectable form.

43. The method of claim 42 wherein the antibody-drug conjugate is administered intravenously.

44. The method of claim 35 wherein the patient is administered a growth inhibitory antibody, in combination with the antibody-drug conjugate compound.

45. The method of claim 35 wherein the patient is administered a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, in combination with the antibody-drug conjugate compound.

46. The method of claim 45 wherein the second antibody comprises monoclonal antibody 2C4 or humanized 2C4.

47. The method of claim 46 wherein said second antibody is conjugated with a cytotoxic agent.

48. The method of claim 35 wherein the patient is administered a chemotherapeutic agent, in combination with the antibody-drug conjugate compound, where the chemotherapeutic agent is selected from Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozole, Imatinib mesylate, PTK787/ZK 222584, Oxaliplatin, 5-FU, Leucovorin, Rapamycin, Lapatinib, Lonafarnib, Sorafenib, and Gefitinib.

49. The method of claim 35 wherein the patient is administered an anti-angiogenic agent in combination with the antibody-drug conjugate compound where the anti-angiogenic agent is selected from bevacizumab.

50. A method of inhibiting the growth of tumor cells that overexpress a growth factor receptor, comprising administering to a patient an antibody-drug conjugate of claim 1 which binds specifically to a growth factor receptor selected from HER2 or EGF and a chemotherapeutic agent wherein said antibody-drug conjugate and said chemotherapeutic agent are each administered in amounts effective to inhibit growth of tumor cells in the patient.

51. A method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor, comprising administering a combination of an antibody-drug conjugate of claim 1 and a chemotherapeutic agent or a growth inhibitory agent.

52. The method of claim 51 wherein the antibody of the antibody-drug conjugate is an anti-ErbB2 antibody.

53. The method of claim 52 wherein the anti-ErbB2 antibody has a biological characteristic of a 4D5 monoclonal antibody.

54. The method of claim 52 wherein the anti-ErbB2 antibody binds essentially the same epitope as a 4D5 monoclonal antibody.

55. The method of claim 52 wherein the anti-ErbB2 antibody is selected from the group consisting of humanized antibodies huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (trastuzumab).

56. An assay for detecting cancer cells comprising:

(a) exposing cells to an antibody-drug conjugate compound of claim 1 or claim 2; and

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

57. The assay of claim 56 wherein the cells are breast tumor cells.

58. The assay of claim 56 wherein the extent of binding is determined by measuring levels of ErbB-encoding nucleic acid by fluorescent in situ hybridization (FISH).

59. The assay of claim 56 wherein the extent of binding is determined by immunohistochemistry (IHC).

60. An article of manufacture comprising

an antibody-drug conjugate compound of claim 1 or claim 2;

a container; and

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

61. The article of manufacture of claim 60 wherein said package insert of label indicates that the compound can be used to treat cancer characterized by the overexpression of an ErbB2 receptor.

62. The article of manufacture of claim 61 wherein the cancer is breast cancer.

63. The article of manufacture of claim 62 wherein the cancer is characterized by the overexpression of an ErbB2 receptor at a 2+ level or above.

64. A method of making an antibody-drug conjugate compound comprising an antibody covalently attached by a linker to one or more maytansinoid drug moieties, the compound having Formula I:

$$Ab–(L–D)_p \qquad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

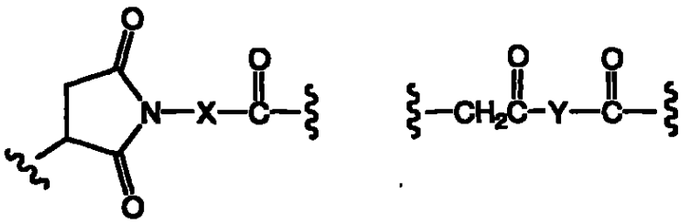
(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

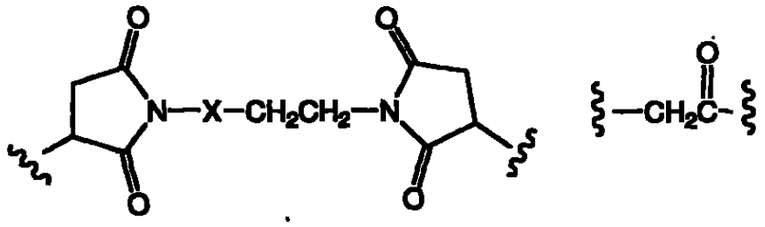
(35) IRTA2 (*Immunoglobulin superfamily receptor translocation associated 2*, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

provided that the antibody is not TA.1;

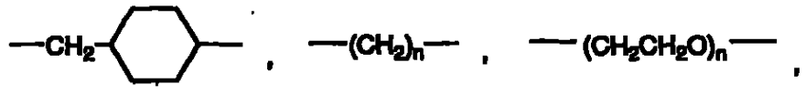
L is a linker selected from the structures;

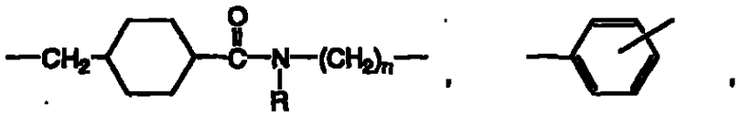


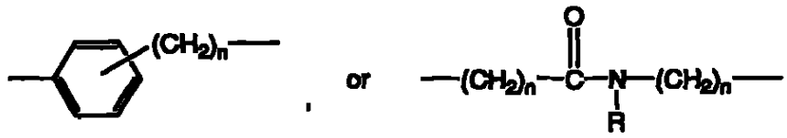


where the wavy lines indicates the covalent attachments to Ab and D;

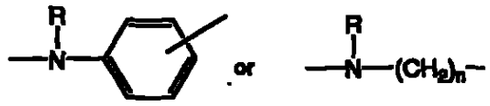
X is:





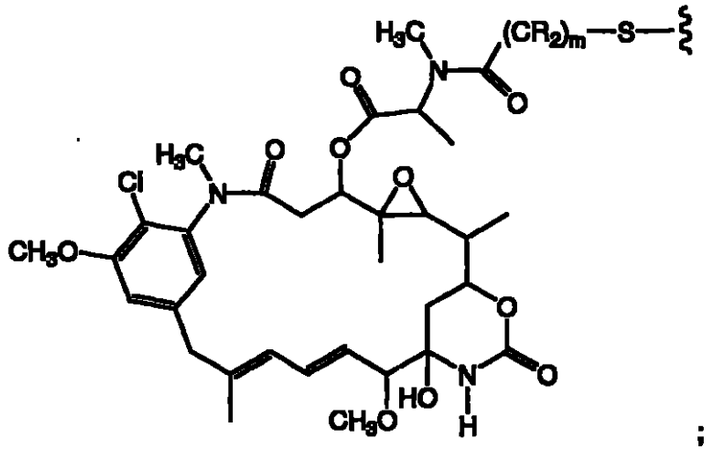


Y is:



where R is independently H or C_1–C_6 alkyl; and n is 1 to 12;

D is a maytansinoid drug moiety selected from the structure:



;

where the wavy line indicates the covalent attachment to L; R is independently H or C_1–C_6 alkyl; m is 1, 2, or 3; and p is 1 to 8;

wherein the method comprises:

reacting Ab with a linker reagent to form antibody-linker intermediate Ab-L, and then reacting Ab-L with a drug moiety D to form the antibody-drug conjugate; or

reacting a drug moiety D with a linker reagent to form a drug-linker intermediate D-L, and then reacting D-L with Ab to form the antibody-drug conjugate.

65. The method of claim 64 wherein Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36).

66. The method of claim 64 or claim 65 wherein the linker reagent is SMCC.

67. The method of claim 64 or claim 65 wherein the linker reagent is a bis-maleimide reagent selected from DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, and $BM(PEO)_4$.

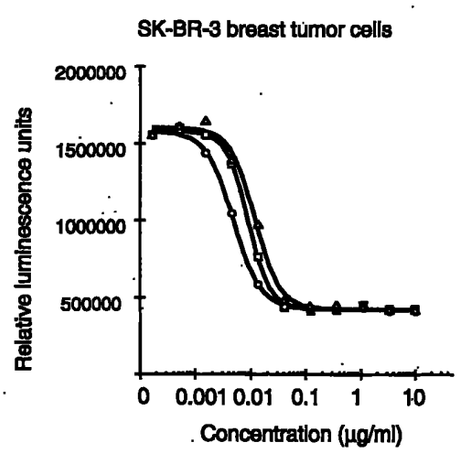


Figure 1

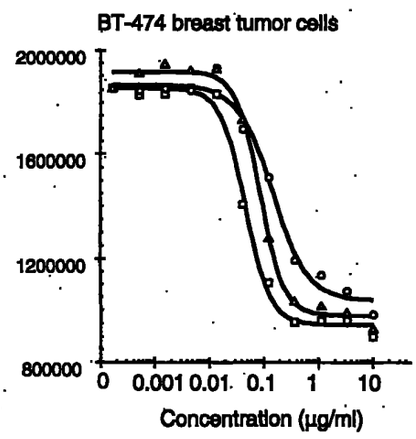


Figure 2

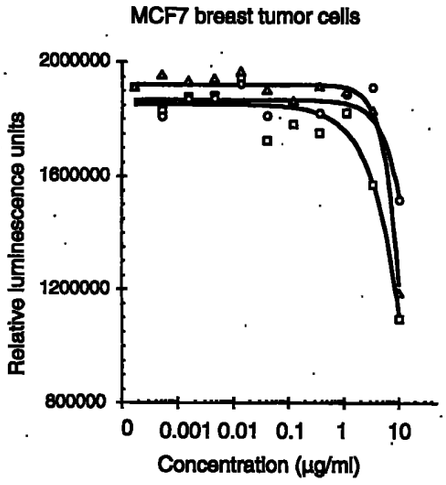


Figure 3

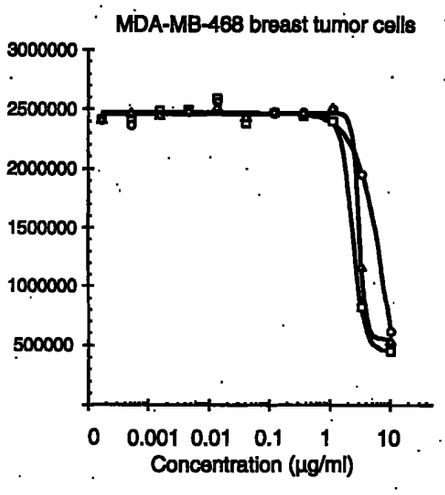


Figure 4

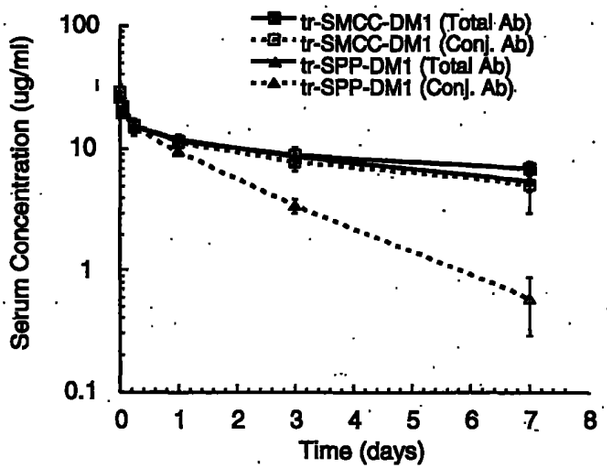

tr-SMCC-DM1 (Total Ab)
tr-SMCC-DM1 (Conj. Ab)
tr-SPP-DM1 (Total Ab)
tr-SPP-DM1 (Conj. Ab)
Serum Concentration (ug/ml)
100
10
1
0.1
0
1
2
3
4
5
6
7
8
Time (days)

Figure 5

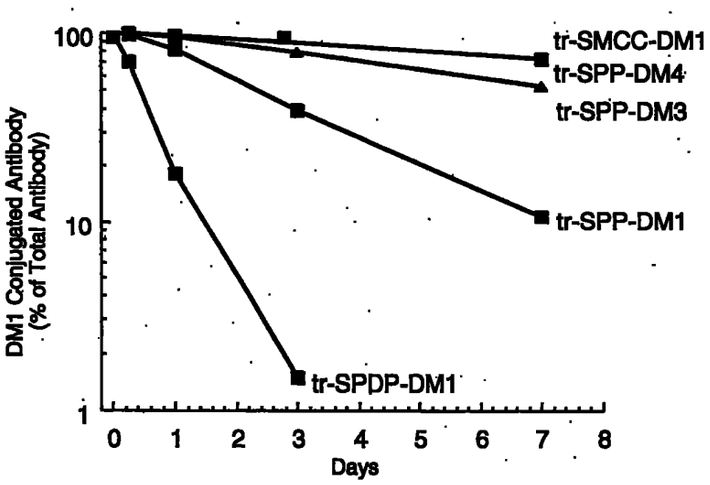

100
10
1
DM1 Conjugated Antibody (% of Total Antibody)
0
1
2
3
4
5
6
7
8
Days
tr-SMCC-DM1
tr-SPP-DM4
tr-SPP-DM3
tr-SPP-DM1
tr-SPDP-DM1

Figure 6

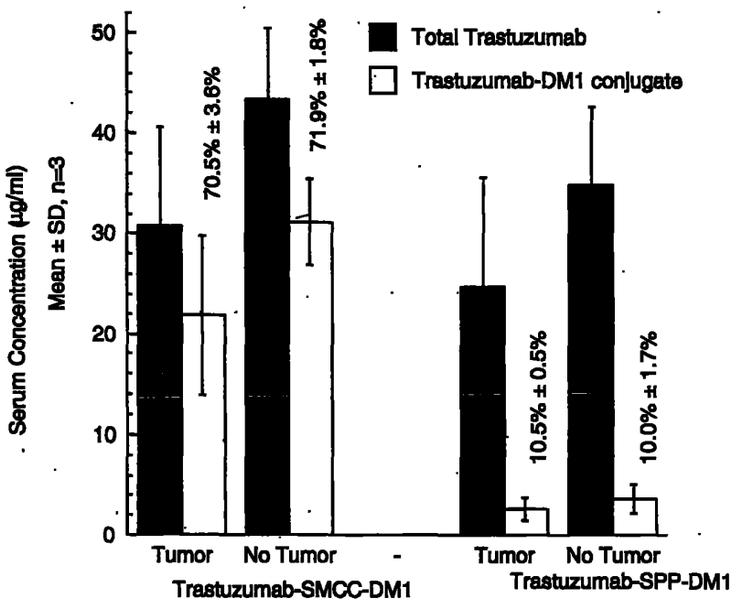

Serum Concentration (µg/ml)
Mean ± SD, n=3
50
40
30
20
10
0
Total Trastuzumab
Trastuzumab-DM1 conjugate
70.5% ± 3.6%
71.9% ± 1.8%
10.5% ± 0.5%
10.0% ± 1.7%
Tumor
No Tumor
-
Tumor
No Tumor
Trastuzumab-SMCC-DM1
Trastuzumab-SPP-DM1

Figure 7

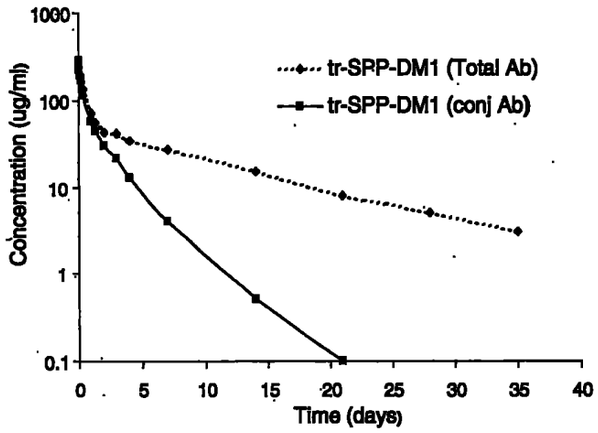

1000
100
10
1
0.1
Concentration (ug/ml)
0
5
10
15
20
25
30
35
40
Time (days)
tr-SPP-DM1 (Total Ab)
tr-SPP-DM1 (conj Ab)

Figure 8

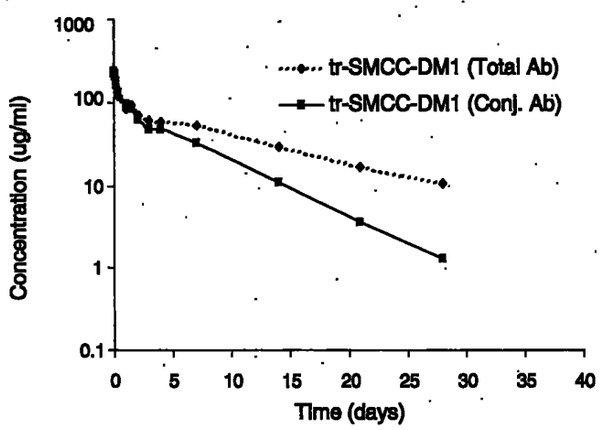

1000
100
10
1
0.1
Concentration (ug/ml)
0
5
10
15
20
25
30
35
40
Time (days)
tr-SMCC-DM1 (Total Ab)
tr-SMCC-DM1 (Conj. Ab)

Figure 9

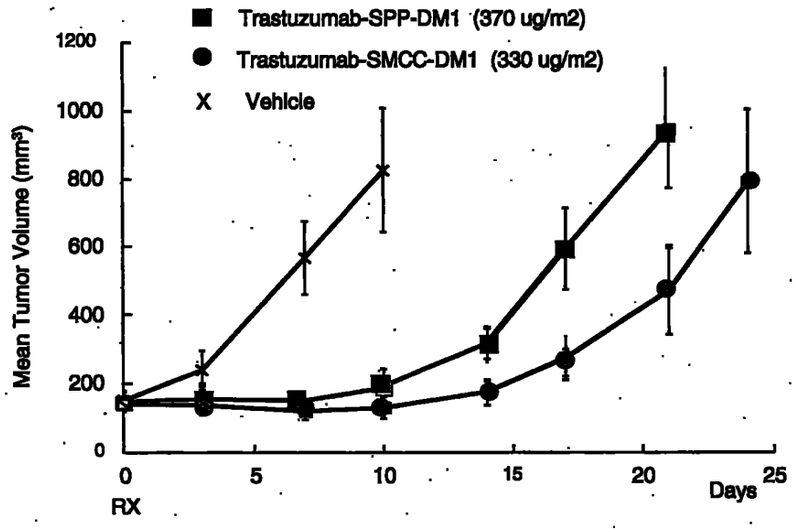

Trastuzumab-SPP-DM1 (370 ug/m2)
Trastuzumab-SMCC-DM1 (330 ug/m2)
X Vehicle
Mean Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Days
RX

Figure 10

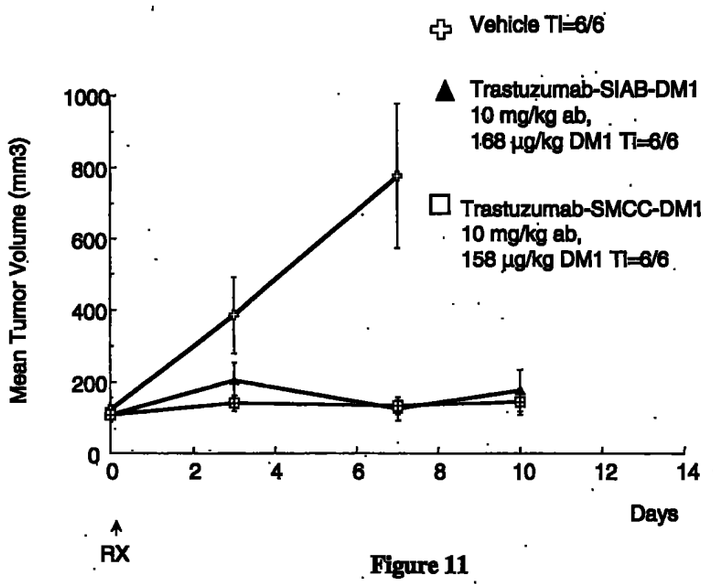

Vehicle TI=6/6
Trastuzumab-SIAB-DM1
10 mg/kg ab,
168 µg/kg DM1 TI=6/6
Trastuzumab-SMCC-DM1
10 mg/kg ab,
158 µg/kg DM1 TI=6/6
Mean Tumor Volume (mm3)
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
Days
RX

Figure 11

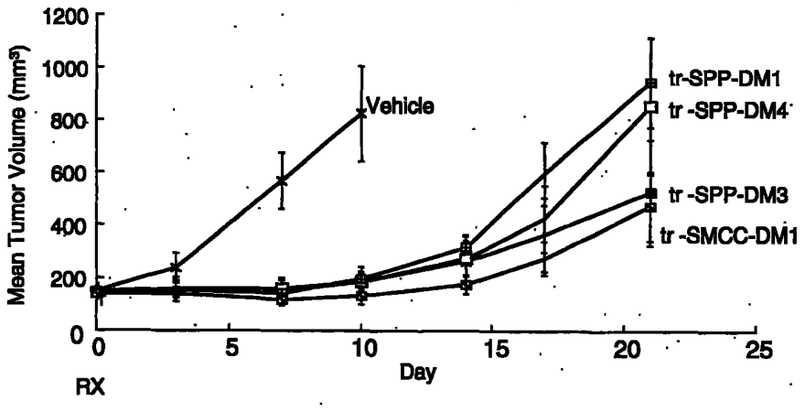
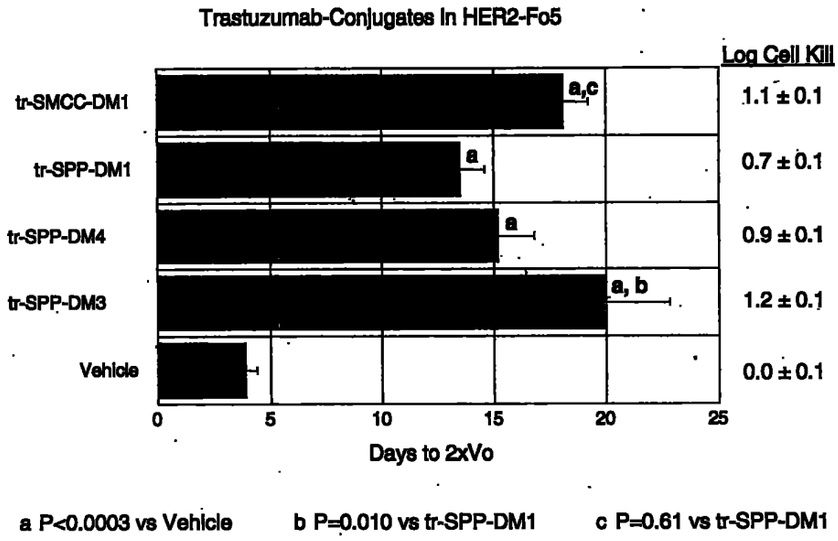

1200
1000
800
600
400
200
0
Mean Tumor Volume (mm³)
Vehicle
tr-SPP-DM1
tr -SPP-DM4
tr -SPP-DM3
tr -SMCC-DM1
0
5
10
15
20
25
Day
RX

Figure 12



a P<0.0003 vs Vehicle b P=0.010 vs tr-SPP-DM1 c P=0.61 vs tr-SPP-DM1

Figure 13

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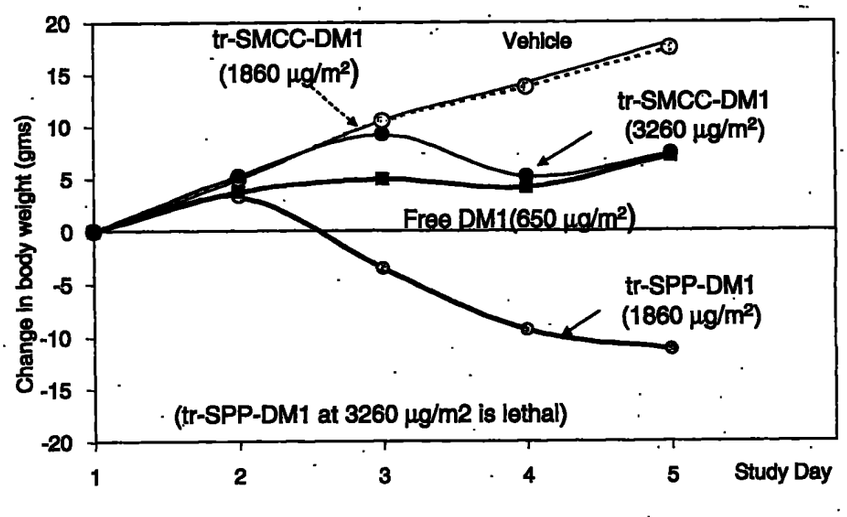
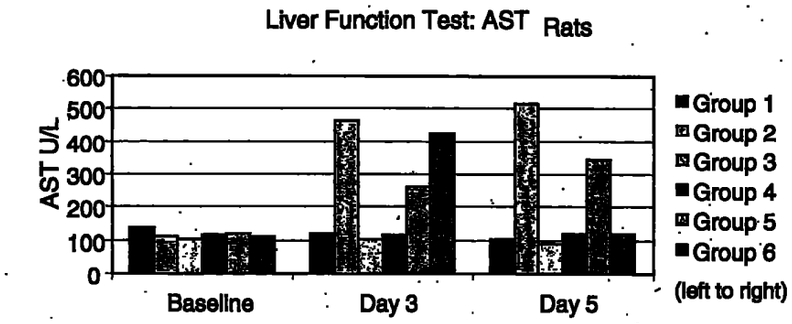
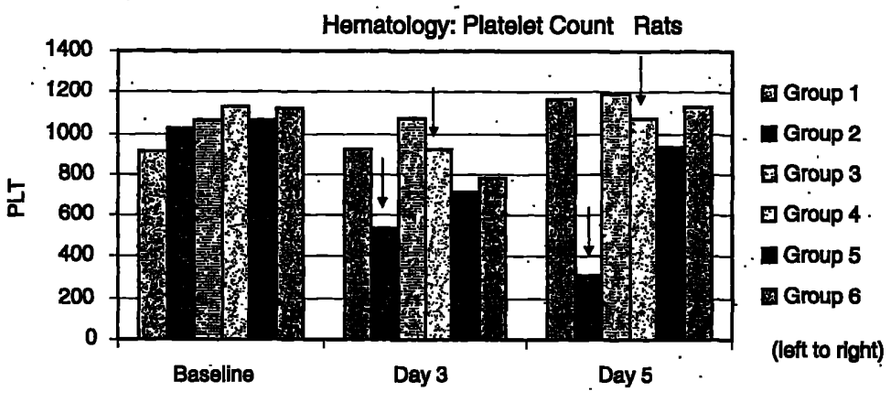

tr-SMCC-DM1
(1860 µg/m²)
Vehicle
tr-SMCC-DM1
(3260 µg/m²)
Free DM1(650 µg/m²)
tr-SPP-DM1
(1860 µg/m²)
(tr-SPP-DM1 at 3260 µg/m2 is lethal)
Change in body weight (gms)
Study Day
20
15
10
5
0
-5
-10
-15
-20
1
2
3
4
5

Figure 14.



Group 1: Vehicle
Group 2: tr-SPP-DM1, 22.3mg/kg
Group 3: tr-SMCC-DM1, 10mg/kg
Group 4: tr-SMCC-DM1, 25mg/kg
Group 5: tr-SMCC-DM1, 50mg/kg
Group 6, free DM1

Figure 15



Group 1: Vehicle
Group 2: tr-SPP-DM1, 22.3mg/kg
Group 3: tr-SMCC-DM1, 10mg/kg
Group 4: tr-SMCC-DM1, 25mg/kg
Group 5: tr-SMCC-DM1, 50mg/kg
Group 6, free DM1

Figure 16

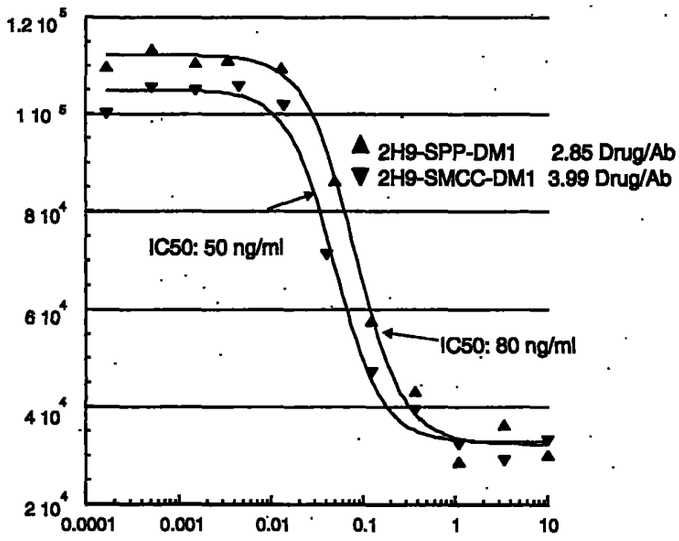

1.2 10^5
1 10^5
8 10^4
6 10^4
4 10^4
2 10^4
0.0001
0.001
0.01
0.1
1
10
2H9-SPP-DM1 2.85 Drug/Ab
2H9-SMCC-DM1 3.99 Drug/Ab
IC50: 50 ng/ml
IC50: 80 ng/ml

Figure 17

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WO 2005/117986 A2

(54) Título: CONJUGADOS DE ANTICUERPO-MEDICAMENTO Y MÉTODOS

(57) Extracto: La presente invención se refiere a compuestos de conjugado anticuerpo-medicamento de Fórmula I: Ab-(L-D)p donde una o más fracciones de medicamento maitansinoide (D) se enlazan covalentemente mediante L a un anticuerpo (Ab), que se une a un receptor ErbB, o que se une a uno o más antígenos asociados con tumores, o receptores de superficie celular. Estos compuestos pueden utilizarse en métodos de diagnóstico o tratamiento de cáncer, y otras enfermedades o trastornos.

CONJUGADOS DE ANTICUERPO-MEDICAMENTO Y MÉTODOS

Esta solicitud no provisional, presentada bajo 37 CFR § 1.53(b), reivindica el beneficio bajo 35 USC §119(e) de la Solicitud Provisional de los Estados Unidos Serie No. 60/576, 517, presentada el 1 de junio de 2004 y Solicitud Provisional de los Estados Unidos Serie No. 60/616, 098, presentada el 5 de octubre de 2004, cada una de las cuales se incorporan por referencia en su integridad.

CAMPO DE LA INVENCIÓN

La invención se refiere generalmente a compuestos con actividad anti-neoplásica y, más específicamente, a anticuerpos conjugados con medicamentos maitansinoides quimioterapéuticos o toxinas. La invención se refiere asimismo a métodos de usar compuestos de conjugado anticuerpo-medicamento para diagnosis *in vitro*, *in situ*, e *in vivo* o tratamiento de células de mamífero, o estados patológicos asociados.

ANTECEDENTES DE LA INVENCIÓN

La terapia con anticuerpo se ha establecido para el tratamiento selectivo de pacientes con cáncer, trastornos inmunitarios y angiogénicos. El uso de conjugados anticuerpo-medicamento (ADC), es decir inmunoconjugados, para la administración local de agentes citotóxicos o citostáticos, es decir medicamentos para eliminar o inhibir células tumorales en el tratamiento de cáncer (Payne, G. (2003) Cancer Cell 3:207-212; Trail et al (2003) Cancer Immunol. Immunother. 52:328-337; Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drug Del. Rev. 26:151-172; US 4975278) permite, en teoría, la aplicación selectiva (***"targeted delivery"***) de la fracción de medicamento a tumores, y la acumulación intracelular en ellos, donde la administración sistémica de estos agentes de medicamento no conjugado puede resultar en niveles inaceptables de toxicidad a las células normales, así como las células tumorales que se busca eliminar (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. (eds), pp.475-506). Con ello se busca la máxima eficacia con una toxicidad mínima. Esfuerzos por diseñar y afinar los ADC se han centrado en la selectividad de anticuerpos monoclonales (mAbs), así como en las propiedades de enlazamiento a medicamentos y liberación del medicamento. Se ha divulgado que los anticuerpos policlonales y los anticuerpos monoclonales son útiles en estas estrategias (Rowland et al (1986) Cancer Immunol. Immunother. 21:183-87). Los medicamentos empleados en estos métodos incluyen daunomicina, doxorubicina, metotrexato, mitomicina, neocarzinostatina (Takahashi et al (1988) Cancer 61:881-888) y vindesina (Rowland et al (1986) *supra*). Las toxinas usadas en conjugados anticuerpo-toxina incluyen toxinas bacterianas tales como toxina de la difteria, toxinas vegetales tales como ricina (US 4753894; US 5629197; US 4958009; US 4956453), toxinas de molécula pequeña tales como geldanamicina (Mandler et al (2000) J. 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), maitansinoides (EP 1391213; Liu et al (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), y caliqueamicina (Lode et al (1998) Cancer Res. 58:2928; Hinman .et al (1993) Cancer Res. 53:3336-3342). Las toxinas pueden producir sus efectos citotóxicos y citostáticos mediante mecanismos tales como inhibición de unión de tubulina, unión de ADN, o topoisomerasa. Algunos medicamentos citotóxicos tienden a ser inactivos, o menos activos, cuando se conjugan con grandes anticuerpos o ligandos de receptor de proteína.

Se ha aprobado un conjugado anticuerpo-radioisótopo, ZBVALIN® (ibritumomab tiuxetan, Biogen/Idec) compuesto de un anticuerpo monoclonal de IgG1 kappa murina, dirigido contra el antígeno CD20 y ^{111}In o ^{90}Y radioisótopo unido por un quelante-enlazador tiourea (Wiseman et al (2000) Eur. J. 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). MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), un conjugado anticuerpo-medicamento compuesto de un anticuerpo huCD33 enlazado a caliqueamicina, fue aprobado en 2000 para el tratamiento de leucemia mieloide mediante inyección (Drugs of the Future (2000) 25(7):686; US 4970198; US 5079233; US 5585089; US 5606040; US 5693762; US 5739116; US 5767285; US 5773001). Cantuzumab mertansina (Immunogen, Inc.), un conjugado anticuerpo-medicamento compuesto del anticuerpo huC242, enlazado mediante el enlazador disulfuro SPP a la fracción de medicamento maitansinoide, DM1 (Xie et al (2004) J. of Pharm. and Exp. Ther. 308(3):1073-1082; Tolcher et al (2003) J. Clin. Oncology 21(2):211-222; US 5208020), fue sometido a estudios clínicos de Fase I para el tratamiento de neoplasias malignas, que expresan CanAg, tales como cáncer de colon, pancreático, gástrico, y otros. MLN-2704 (Millennium Pharm., BZL Biologies, Immunogen Inc.) es un conjugado anticuerpo-medicamento compuesto del anticuerpo monoclonal de antígeno específico de membrana anti-próstata (PMSA), enlazado a la fracción de medicamento maitansinoide, DM1, bajo desarrollo para el tratamiento potencial de tumores de la próstata. La misma fracción de medicamento maitansinoide, DM1, se enlazó mediante un enlazador no-disulfuro, SMCC, a un anticuerpo monoclonal murino en ratón, TA.1 (Chari et al. (1992) Cancer Research 52:127-131). Se informó que este conjugado era 200 veces menos potente que el conjugado de enlazador disulfuro correspondiente. Se consideró allí que el enlazador SMCC era "no segmentable" (ver también: US 4981979). Se ha divulgado HERCEPTIN® (trastuzumab) enlazado por SMCC a DM1 (WO 2005/037992).

En intentos por descubrir dianas celulares efectivas para diagnosis y terapia de cáncer, los investigadores han buscado identificar polipéptidos de transmembrana o asociados de algún otro modo con un tumor, que se expresan especificamente en la superficie de uno o más tipos particulares de célula cancerosa, comparados con en la superficie de una o más células no

cancerosas normales. A menudo, tales polipéptidos asociados con tumores se expresan más abundantemente en la superficie de las células cancerosas, comparados con en la superficie de las células no cancerosas. La identificación de tales polipéptidos de antígeno de superficie celular asociados con tumores, es decir antígenos asociados con tumores (TAA), ha dado lugar a la capacidad de seleccionar específicamente células cancerosas para destrucción, mediante terapias a base de anticuerpos.

La terapia de anticuerpo monoclonal se ha establecido para el tratamiento selectivo de pacientes con cáncer, trastornos inmunitarios y angiogénicos. Un ejemplo de terapia exitosa de anticuerpo es HERCEPTIN® (trastuzumab), un anticuerpo monoclonal humanizado derivado de ADN recombinante, que se une selectivamente con alta afinidad, en un ensayo a base de células (Kd = 5 nM), al dominio extracelular de la proteína receptor 2 del factor de crecimiento epidérmico humano, HER2 (ErbB2) (US 5821337; US 6054297; US 6407213; US 6639055; Coussens L, et al (1985) Science 230:1132-9; Slamon DJ, et al (1989) Science 244:707-12). Trastuzumab es un anticuerpo IgG1 kappa que contiene regiones marco humanas con las regiones determinantes de complementaridad de un anticuerpo murino (4D5), que se une a HER2. Trastuzumab se une al antígeno HER2 y, por consiguiente, inhibe el crecimiento de células cancerosas. Debido a que trastuzumab es un anticuerpo humanizado, éste minimiza cualquier respuesta HAMA en pacientes. El anticuerpo humanizado contra HER2 se produce por un cultivo en suspensión de célula de mamífero (Ovario de Hámster de China, CHO). El proto-oncogén HER2 (ó c-erbB2) codifica una proteína receptor de transmembrana de 185kDa, que se relaciona estructuralmente con el receptor de factor de crecimiento epidérmico. La sobreexpresión de la proteína HER2 se observa en 25%-30% de las neoplasias malignas de mama primarias y puede determinarse usando una evaluación basada en inmunohistoquímica de bloques tumorales (Press MF, et al (1993) Cancer Res 53:4960-70. Se ha demostrado que el trastuzumab, tanto en ensayos *in vitro* como en animales, inhibe la proliferación de células tumorales

humanas que sobreexpresan HER2 (Hudziak RM, et al (1989) Mol Cell Biol 9:1165-72; Lewis GD, et al (1993) Cancer Immunol Immunother; 37:255-63; Baselga J, et al (1998) Cancer Res. 58:2825-2831). El trastuzumab es un mediador de la citotoxicidad celular dependiente de anticuerpo, ADCC (Hotaling TE, et al (1996) [abstract]. Proc. Annual Meeting Am Assoc Cancer Res; 37:471; Pegram MD, et al (1997) [abstract]. Proc Am Assoc Cancer Res; 38:602; Sliwkowski et al (1999) Seminars in Oncology 26(4), Suppl 12:60-70; Yarden Y. and Sliwkowski, M. (2001) Nature Reviews: Molecular Cell Biology, Macmillan Magazines, Ltd., Vol. 2: 127-137). HERCEPTIN® es clínicamente activo en pacientes con neoplasias malignas de mama metastásicas, que sobreexpresan ErbB2, los cuales han recibido terapia antineoplásica previa extensiva (Baselga et al, (1996) J. Clin. Oncol. 14:737-744). Aunque el HERCEPTIN constituye un gran adelanto en tratar pacientes con neoplasias malignas de mama que sobreexpresan ErbB2, los cuales han recibido terapia antineoplásica previa extensiva, la mayoría de los pacientes en esta población no responden o responden sólo a medias al tratamiento con HERCEPTIN. Por lo tanto, subsiste una necesidad clínica significativa para desarrollar terapias adicionales contra el cáncer, dirigidas a HER2, para aquellos pacientes con tumores que sobreexpresan HER2 u otras enfermedades asociadas con la expresión de HER2, que no responden, o responden a medias, al tratamiento con HERCEPTIN. Aparte del HER2, existe una oportunidad de explotar otros antígenos, asociados con tumores, con terapias selectivas.

SUMARIO

La presente invención provee compuestos novedosos con actividad biológica contra células cancerosas. Los compuestos pueden inhibir el crecimiento tumoral en mamíferos y pueden ser útiles para tratar pacientes humanos con cáncer. La presente invención se refiere a la administración, transporte, acumulación o retención de compuestos terapéuticos de conjugado anticuerpo-medicamento (ADC) dentro de las células. La invención se relaciona más particularmente con obtener altas

concentraciones de moléculas de metabolito activo en células cancerosas. La selectividad intracelular ("***intracellular targeting***") puede lograrse por métodos y compuestos que permitan la acumulación o retención de agentes biológicamente activos dentro de las células. Tal selectividad efectiva puede ser aplicable a una variedad de formulaciones y procedimientos terapéuticos.

Se ha hecho el sorprendente descubrimiento de que conjugados anticuerpo-medicamento con grupos enlazadores no-disulfuro, estables, que fijan una fracción de medicamento maitansinoide a un anticuerpo se traducen en una mayor potencia *in vitro* y eficacia *in vivo*. Además, los conjugados anticuerpo-medicamento muestran el resultado inesperado de una mejor seguridad *in vivo*, en relación con ciertos conjugados de enlazador disulfuro.

Los compuestos de conjugado anticuerpo-medicamento (ADC) comprenden un anticuerpo unido covalentemente por un enlazador a una o más fracciones de medicamento maitansinoide. Los ADC pueden representarse por la Fórmula I:

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

en donde una o más fracciones de medicamento maitansinoide (D) se enlazan covalentemente por L a un anticuerpo (Ab). Ab es un anticuerpo que se une a un receptor ErbB, o que se une a uno o más antígenos asociados con tumores o receptores de superficie celular. El enlazador L puede ser estable fuera de una célula, es decir extracelular. El enlazador L, la fracción de medicamento maitansinoide D, o el enlazador y la fracción de medicamento maitansinoide, tomados conjuntamente, (L-D), no comprenden un grupo disulfuro.

En una incorporación, una cantidad sustancial de la fracción de medicamento no se separa del anticuerpo hasta cuando el conjugado anticuerpo-medicamento penetra una célula con un receptor de superficie celular específico para el anticuerpo del conjugado anticuerpo-

medicamento, y la fracción de medicamento se separa del anticuerpo cuando el conjugado anticuerpo-medicamento penetra la célula.

En otra incorporación, el ADC se une específicamente a un receptor codificado por un gen ErbB, tal como EGFR, HER2, HER3 y HER4. El ADC puede unirse específicamente al dominio extracelular del receptor HER2. El ADC puede inhibir el crecimiento de células tumorales que sobreexpresan el receptor HER2.

En otra incorporación, el anticuerpo (Ab) de Fórmula I es un anticuerpo humanizado tal como huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 ó huMAb4D5-8 (trastuzumab).

Otro aspecto de la invención es una composición farmacéutica que incluye un compuesto de Fórmula I, o su sal o solvato farmacéuticamente aceptable, y un diluyente, portador o excipiente farmacéuticamente aceptable.

Otro aspecto provee una combinación farmacéutica, que comprende un compuesto de Fórmula I, y un segundo compuesto con propiedades antineoplásicas u otros efectos terapéuticos.

Otro aspecto incluye usos diagnósticos y terapéuticos para los compuestos y composiciones divulgados en esta solicitud.

Otro aspecto es un método para eliminar o inhibir la proliferación de células tumorales o células cancerosas, el cual comprende tratar las células con una cantidad de un conjugado anticuerpo-medicamento, o su sal o solvato farmacéuticamente aceptable, que es efectivo para eliminar o inhibir la proliferación de las células tumorales o células cancerosas.

Otro aspecto son métodos de tratar cáncer, los cuales comprenden administrar a un paciente una formulación de un compuesto de Fórmula I. Un método es para el tratamiento de cáncer en un mamífero, en donde el cáncer se caracteriza por la sobreexpresión de un receptor ErbB. Opcionalmente el mamífero no responde, o responde a medias, al tratamiento con un anticuerpo anti-ErbB no conjugado. El método

comprende administrar al mamífero una cantidad terapéuticamente efectiva de un compuesto de conjugado anticuerpo-medicamento.

Otro aspecto es un método de inhibir el crecimiento de células tumorales que sobreexpresan un receptor de factor de crecimiento, seleccionado del grupo conformado por el receptor HER2 y el receptor EGF, método que comprende administrar a un paciente un compuesto conjugado anticuerpo-medicamento, que se une específicamente a dicho receptor de factor de crecimiento, y un agente quimioterapéutico, en donde dicho conjugado anticuerpo-medicamento y dicho agente quimioterapéutico se administran cada uno en cantidades efectivas para inhibir el crecimiento de células tumorales en el paciente.

Otro aspecto es un método para el tratamiento de un paciente humano susceptible a, o diagnosticado con, un trastorno caracterizado por sobreexpresión de receptor ErbB2, método que comprende administrar una combinación de un compuesto de conjugado anticuerpo-medicamento de Fórmula.I y un agente quimioterapéutico.

Otro aspecto es un método de análisis para detectar células cancerosas, el cual comprende: exponer las células a un compuesto de conjugado anticuerpo-medicamento, y determinar la magnitud de unión a las células del compuesto de conjugado anticuerpo-medicamento.

Otro aspecto concierne a métodos de cribar candidatos medicinales ADC para el tratamiento de una enfermedad o trastorno, donde la enfermedad o trastorno se caracteriza por la sobreexpresión de HER2.

Otro aspecto incluye artículos de fabricación, es decir kits, que comprenden un conjugado anticuerpo-medicamento, un recipiente, y un prospecto de envase o rótulo indicando un tratamiento.

Otro aspecto incluye métodos de tratar una enfermedad o trastorno, caracterizado por la sobreexpresión de HER2 en un paciente, con los compuestos de conjugado anticuerpo-medicamento.

Otro aspecto incluye métodos de elaborar, métodos de preparar, métodos de síntesis, métodos de conjugación, y métodos de purificación de los compuestos de conjugado anticuerpo-medicamento, y los intermedios

para la preparación, síntesis y conjugación de los compuestos de conjugado anticuerpo-medicamento.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

Figura 1 muestra un análisis de proliferación celular *in vitro* con células SK-BR-3 tratadas con conjugados anticuerpo-medicamento: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, y –o– trastuzumab-SMCC-DM1.

Figura 2 muestra un análisis de proliferación celular *in vitro* con células BT-474 tratadas con conjugados anticuerpo-medicamento: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, y –o– trastuzumab-SMCC-DM1.

Figura 3 muestra un análisis de proliferación celular *in vitro* con células MCF7 tratadas con conjugados anticuerpo-medicamento: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, y –o– trastuzumab-SMCC-DM1.

Figura 4 muestra un análisis de proliferación celular *in vitro* con células MDA-MB-468 tratadas con conjugados anticuerpo-medicamento: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, y –o– trastuzumab-SMCC-DM1.

Figura 5 muestra la depuración en ratones lampiños beige, sin tumores, de trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, midiendo la concentración sérica de anticuerpo total y conjugado en seis puntos de tiempo (5 minutos, 1 hora, 6 horas, 24 horas, 72, 168 horas pos-dosis) durante 7 días.

Figura 6 muestra la estabilidad en el tiempo en ratones lampiños, sin tumores, de los conjugados: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, y trastuzumab-SMCC- DM1, midiendo la concentración sérica en seis puntos de tiempo (5 minutos, 1 hora, 6 horas, 24 horas, 72, 168 horas pos-dosis) durante 7 días.

Figura 7 muestra la medición de concentraciones séricas de trastuzumab total / trastuzumab-SMCC-DM1, y trastuzumab total / trastuzumab-SPP-DM1 en ratones, 7 días después del tratamiento, con y sin tumores.

Figura 8 muestra un estudio de depuración de concentración plasmática luego de la administración de 10 mg/kg de trastuzumab-SPP-DM1 a 4 sujetos ratas. Se midieron las concentraciones de anticuerpo total y trastuzumab-SPP-DM1, (tr = trastuzumab).

Figura 9 muestra un estudio de depuración de concentración plasmática luego de la administración de 10 mg/kg de trastuzumab-SMCC-DM1 a 4 sujetos ratas. Se midieron las concentraciones de anticuerpo total y trastuzumab-SMCC-DM1.

Figura 10 muestra el cambio en volumen medio del tumor en el tiempo en ratones medicados con: Vehículo (PBS pH 6.5), trastuzumab-SPP-DM1 (370 μg DM1/m^2), y trastuzumab-SMCC-DM1 (330 μg DM1/m^2), en donde dosis se refiere a la dosis de DM1 administrado.

Figura 11 muestra el cambio en volumen medio del tumor en el tiempo en ratones lampiños atímicos con aloinjertos de tumor Fo5, medicados el Día 0 con: Vehículo (PBS pH 6.5), 10 mg/kg trastuzumab-SIAB-DM1 (3.4 DM1/Ab; 168 μg DM1/kg), y 10 mg/kg trastuzumab-SMCC-DM1 (3.2 DM1/Ab; 158 μg DM1/kg), en donde dosis se refiere a la cantidad de dosis del conjugado anticuerpo-medicamento administrado.

Figura 12 muestra el cambio en volumen medio del tumor en el tiempo en ratones lampiños beige MMTV-Her2 Fo 5 (siete en cada grupo, todos con tumores, Ti = 7) mediante inyección simple con Vehículo (PBS pH 6.5), 10 mg/kg trastuzumab- SPP-DM1, 10 mg/kg trastuzumab-SPP-DM4, 10 mg/kg trastuzumab-SPP-DM3, y 10 mg/kg trastuzumab-SMCC-DM1.

Figura 13 muestra el tiempo para doblar el volumen del tumor y el análisis logarítmico de eliminación de células (***"log cell kill analysis"***) para Vehículo (PBS pH 6.5), trastuzumab-SPP-DM1, trastuzumab-SPP-

DM4, trastuzumab-SPP-DM3, y trastuzumab-SMCC-DM1 en tumores HER2-Fo5.

Figura 14 muestra el cambio en peso corporal, en el tiempo, de ratas medicadas con: Vehículo (10 mM succinato de sodio, 100 mg/mL sacarosa, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (3260 μg DM1/m2), y DM1 libre (650 μg/m2).

Figura 15 muestra una prueba de función hepática, medida en unidades AST por litro en el tiempo, en el modelo de rata medicada con: Vehículo (10 mM succinato de sodio, 100 mg/mL sacarosa, 0.1% Tween 20, pH 5.0), trastuzumab-SPP- DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), y DM1 libre.

Figura 16 muestra un perfil de seguridad, medido en unidades PLT en células por litro en el tiempo, en el modelo de rata medicada con: Vehículo (10 mM succinato de sodio, 100 mg/mL sacarosa, 0.1% Tween 20, pH 5.0), trastuzumab-SPP- DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), y DM1 libre.

Figura 17 muestra un ensayo de proliferación celular *in vitro* con células HT1080EphB2 (C8), tratadas con conjugados anticuerpo-medicamento: −▲− antiEphB2R 2H9-SPP-DM1, y −▼− antiEphB2R 2H9-SMCC-DM1.

DESCRIPCIÓN DETALLADA DE INCORPORACIONES EJEMPLARES

Se hará ahora referencia en detalle a ciertas incorporaciones de la invención, cuyos ejemplos se ilustran en las estructuras y fórmulas acompañantes. Aunque la invención se describirá en conjunción con las incorporaciones enumeradas, se entenderá que éstas no pretenden limitar la

invención a estas incorporaciones. Por el contrario, la invención tiene como finalidad cubrir todas las alternativas, modificaciones y equivalentes, que puedan incluirse en el ámbito de la presente invención, como se define por las reivindicaciones.

Un experto en el arte reconocerá muchos métodos y materiales similares o equivalentes a los descritos en esta solicitud, que podrían usarse en la práctica de la presente invención. De ningún modo la presente invención se limita a los métodos y materiales descritos.

A menos que se defina lo contrario, los términos técnicos y científicos incluidos en esta solicitud tienen el mismo significado que entiende una persona de habilidad corriente en el arte, al cual pertenece esta invención, y son consistentes con: Singleton *et al* (1994) *Dictionary of Microbiology and Molecular Biology, 2nd Ed.*, J. Wiley & Sons, New York, NY; y Janeway, C, Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York.

DEFINICIONES

A menos que se indique lo contrario, la finalidad de los siguientes términos y expresiones, tal como se emplean en esta solicitud, es tener los siguientes significados:

Cuando se emplean nombres comerciales en esta solicitud, los solicitantes tienen la intención de incluir independientemente la formulación del producto de marca registrada, el medicamento genérico y el ingrediente(s) farmacéutico activo del producto de marca registrada.

El término "anticuerpo" en esta solicitud se emplea en el sentido más amplio y cubre específicamente anticuerpos monoclonales, anticuerpos policlonales, dímeros, multímeros, anticuerpos multiespecíficos (*por ejemplo*, anticuerpos bioespecíficos), y fragmentos de anticuerpo, siempre y cuando exhiban la actividad biológica deseada (Miller et al (2003) Jour. of Immunology 170:4854-4861). Los anticuerpos pueden ser murinos, humanos, humanizados, quiméricos o derivados de otras especies. Un anticuerpo es una proteína generada por el sistema inmunitario, que es

capaz de reconocer y unirse a un antígeno específico. (Janeway, C, Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York). Un antígeno selectivo tiene generalmente numerosos puntos de unión, también llamados epítopos, reconocidos por CDR en múltiples anticuerpos. Cada anticuerpo que específicamente se une a un epítopo diferente posee una estructura diferente. Por consiguiente, un antígeno puede tener más de un anticuerpo correspondiente. Un anticuerpo incluye una molécula de inmunoglobulina de cadena completa o una porción inmunológicamente activa de una molécula de inmunoglobulina de cadena completa, es decir, una molécula que contiene un sitio de unión de antígeno, que inmunoespecíficamente une un antígeno de una diana de interés o parte del mismo, incluyendo tales dianas, pero sin limitarse a, célula o células cancerosas, que produzcan anticuerpos autoinmunitarios asociados con una enfermedad autoinmunitaria. La inmunoglobulina divulgada en esta solicitud puede ser de cualquier tipo (*por ejemplo*, IgG, IgE, IgM, IgD, e IgA), clase (*por ejemplo*, IgG1, IgG2, IgG3, IgG4, IgA1 e IgA2) o subclase de molécula de inmunoglobulina. Las inmunoglobulinas pueden derivarse de cualquier especie. En un aspecto, sin embargo, la inmunoglobulina es de origen humano, murino, o de conejo.

"Fragmentos de anticuerpo" comprenden una porción de un anticuerpo de cadena completa, generalmente su región variable o de unión de antígeno. Ejemplos de fragmentos de anticuerpo incluyen fragmentos Fab, Fab', $F(ab')_2$, Fv; diacuerpos; anticuerpos lineales; fragmentos producidos por una biblioteca de expresión Fab, anticuerpos anti-idiotípicos (anti-Id), CDR (región determinante de complementaridad), y fragmentos de unión de epítopo de cualquiera de los anteriores, que se unan inmunoespecíficamente a antígenos de célula cancerosa, antígenos víricos o antígenos microbianos, moléculas de anticuerpo de cadena sencilla; y anticuerpos multispecíficos formados de fragmentos de anticuerpo.

El término "anticuerpo monoclonal", como se emplea en esta solicitud, se refiere a un anticuerpo obtenido de una población de anticuerpos sustancialmente homogéneos, *es decir*, los anticuerpos

individuales que componen la población son idénticos, excepto por posibles mutaciones de ocurrencia natural, que puedan estar presentes en cantidades menores. Los anticuerpos monoclonales son altamente específicos, dirigiéndose contra un solo sitio antigénico. Más aún, en contraste con preparaciones de anticuerpo policlonal, que incluyen diferentes anticuerpos dirigidos contra diferentes determinantes (epítopos), cada anticuerpo monoclonal se dirige contra un solo determinante en el antígeno. Además de su especificidad, los anticuerpos monoclonales son ventajosos en el sentido de que pueden sintetizarse incontaminados por otros anticuerpos. El calificativo "monoclonal" indica el carácter del anticuerpo como obtenido de una población sustancialmente homogénea de anticuerpos, y no tiene que interpretarse como que requiere la producción del anticuerpo por algún método particular. Por ejemplo, los anticuerpos monoclonales a utilizarse de acuerdo con la presente invención pueden elaborarse por el método del hibridoma, descrito por primera vez por Kohler *et al.* (1975), *Nature,* 256: 495, o pueden elaborarse por métodos de ADN recombinante (véase, *por ejemplo*, US. 4816567). Los anticuerpos monoclonales pueden aislarse también de bibliotecas de anticuerpos de fagos usando las técnicas descritas en Clackson *et al.* (1991), *Nature*, 352:624-628; Marks *et al.* (1991), *J. Mol. Biol.*, 222:581-597, por ejemplo.

Los anticuerpos monoclonales en esta solicitud incluyen específicamente anticuerpos "quiméricos", en los cuales una porción de la cadena pesada y/o ligera es idéntica con, u homóloga a, secuencias correspondientes en anticuerpos derivados de una especie particular, o pertenecientes a una clase o subclase de anticuerpos, mientras que el resto de la cadena (s) es idéntico con, u homólogo a, secuencias correspondientes en anticuerpos derivados de otra especie, o pertenecientes a otra clase o subclase de anticuerpos, así como fragmentos de tales anticuerpos, siempre que exhiban la actividad biológica deseada (US 4816567; y Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855). Los anticuerpos quiméricos de interés en esta solicitud incluyen anticuerpos "primatizados", que comprenden secuencias de unión de antígeno de dominio variable,

derivados de un primate no humano (*por ejemplo*, mono del Viejo Mundo o macaco) y secuencias de región constante humanas.

Un "anticuerpo intacto" en esta solicitud es uno que comprende cominios VL y VH, así como un dominio constante de cadena ligera (CL) y dominios constantes de cadena pesada, CH1, CH_2 y CH3. Los dominios constantes pueden ser dominios constantes de secuencia nativa (*por ejemplo*, dominios constantes de secuencia nativa humana) o su variante de secuencia de aminoácidos. El anticuerpo intacto puede tener una o más "funciones efectoras", las cuales hacen referencia a aquellas actividades biológicas atribuibles a la región Fc (una región Fc de secuencia nativa o una región Fc variante de secuencia de aminoácidos) de un anticuerpo. Ejemplos de funciones efectoras de anticuerpo incluyen unión C1q; citotoxicidad dependiente de complemento; unión de receptor Fc; citotoxicidad celular dependiente de anticuerpo (ADCC); fagocitosis; y regulación por disminución de receptores de superficie celular tales como receptor de linfocito B y BCR.

Dependiendo de la secuencia de aminoácidos del dominio constante de sus cadenas pesadas, los anticuerpos intactos pueden asignarse a diferentes "clases". Existen cinco clases principales de anticuerpos intactos: IgA, IgD, IgE, IgG, e IgM, y varios de estos puede subdividirse en "subclases" (isotipos), *por ejemplo*, IgG1, IgG2, IgG3, IgG4, IgA, e IgA2. Los dominios constantes de cadena pesada, que corresponden a las diferentes clases de anticuerpos, se denominan α, δ, ε, γ y μ, respectivamente. Las estructuras subunitarias y configuraciones tridimensionales de diferentes clases de inmunoglobulinas son bien conocidas.

Un "receptor ErbB" es una proteína tirosina cinasa de receptor, que pertenece a la familia de receptor ErbB, los cuales son mediadores importantes de crecimiento, diferenciación y supervivencia celular. La familia de receptor ErbB comprende cuatro miembros distintos, incluido el receptor del factor de crecimiento epidérmico (EGFR, ErbB1, HER1), HER2 (ErbB2 ó $p185^{neu}$), HER3 (ErbB3) y HER4 (ErbB4 ó tyro2). Se ha

caracterizado un panel de anticuerpos anti-ErbB2, usando la línea de células tumorales de mama humanas SKBR3 (Hudziak et al (1989) Mol. Cell. Biol. 9(3): 1165-1172. Se obtuvo inhibición máxima con el anticuerpo denominado 4D5, el cual inhibió la proliferación celular en un 56%. Otros anticuerpos en el panel redujeron la proliferación celular en una medida menor en este ensayo. Se descubrió además que el anticuerpo 4D5 sensibiliza líneas de células de tumor de mama, que sobreexpresan ErbB2, a los efectos citotóxicos de TNF-α (US 5677171). Los anticuerpos anti-ErbB2, discutidos en Hudziak et al., se caracterizan también en Fendly et al (1990) Cancer Research 50:1550-1558; Kotts et al (1990) In Vitro 26(3):59A; Sarup et al (1991) Growth Regulation 1:72-82; Shepard et al., J. (1991) Clin. Immunol. 11(3):117-127; Kumar et al (1991) Mol. Cell. Biol. 11(2):979-986; Lewis et al (1993) Cancer Immunol. Immunother. 37:255-263; Pietras et al (1994) Oncogene 9:1829-1838; Vitetta et al (1994) Cancer Research 54:5301-5309; Sliwkowski et al (1994) J. Biol. Chem. 269(20):14661-14665; Scott et al (1991) J. Biol. Chem. 266:14300-5; D'souza et al., Proc. Natl. Acad. Sci. (1994) 91:7202-7206; Lewis et al (1996) Cancer Research 56:1457-1465; y Schaefer et al (1997) Oncogene 15:1385-1394.

Otros anticuerpos anti-ErbB2 con diferentes propiedades se han descrito en Franklin et al (2004) Cancer Cell 5:317-328; Tagliabue et al (1991) Int. J. Cancer 47:933-937; McKenzie et al (1989) Oncogene 4:543-548; Maier et al (1991) Cancer Res. 51:5361-5369; Bacus et al (1990) Molecular Carcinogenesis 3:350-362; Stancovski et al (1991) PNAS (USA) 88:8691-8695; Bacus et al (1992) Cancer Research 52:2580-2589; Xu et al (1993) Int. J. Cancer 53:401-408; WO94/00136; Kasprzyk et al (1992) Cancer Research 52:2771-2776; Hancock et al (1991) Cancer Res. 51:4575-4580; Shawver et al (1994) Cancer Res. 54:1367-1373; Arteaga et al (1994) Cancer Res. 54:3758-3765; Harwerth et al (1992) J. Biol. Chem. 267:15160-15167; US 5783186; y Klapper et al (1997) Oncogene 14:2099-2109.

El cribado de identidad de secuencias ha resultado en la identificación de otros dos miembros de la familia de receptor ErbB; ErbB3 (US 5,183,884; US 5,480,968; Kraus et al (1989) PNAS (USA) 86:9193-9197) y ErbB4 (EP 599274; Plowman et al (1993) Proc. Natl. Acad. Sci. USA, 90:1746-1750; y Plowman et al (1993) Nature 366:473-475). Estos dos receptores despliegan una expresión incrementada en por lo menos algunas líneas de células de cáncer de mama.

El receptor ErbB comprenderá generalmente un dominio extracelular, el cual puede unir un ligando ErbB; un dominio trasmembrana lipófilo; un dominio tirosina cinasa intracelular conservado; y un dominio señalador de terminal carboxilo, que albergue varios residuos de tirosina, los cuales pueden forforilarse. El receptor ErbB puede ser un receptor ErbB de "secuencia nativa" o su "variante de secuencia de aminoácidos". El receptor ErbB puede ser un receptor ErbB humano de secuencia nativa. Por consiguiente, un "miembro de la familia de receptor ErbB" es EGFR (ErbB1), ErbB2, ErbB3, ErbB4 o cualquier otro receptor ErbB actualmente conocido, o por identificarse en el futuro.

Los términos "ErbB1", "receptor de factor de crecimiento epidérmico", "EGFR" y "HER1" se emplean en esta solicitud de manera intercambiable y se refieren a EGFR como se divulga, por ejemplo, en Carpenter et al (1987) Ann. Rev. Biochem. 56:881-914, incluidas sus formas mutantes de ocurrencia natural (*por ejemplo*, un EGFR mutante de eliminación como en Humphrey et al (1990) PNAS (USA), 87:4207-4211). El término erbB1 se refiere al gen que codifica el producto proteína EGFR. Anticuerpos contra HER1 se describen, por ejemplo, en Murthy et al (1987) Arch. Biochem. Biophys., 252:549-560 y en WO 95/25167.

El término "ERRP", "Proteína Relacionada con Receptor EGF", "Proteína Relacionada con EGFR" y "proteína relacionada con receptor de factor de crecimiento epidérmico" se emplean en forma intercambiable en esta solicitud y se refieren a ERRP como se divulga, por ejemplo en US 6399743 y US 2003/0096373.

Las expresiones "ErbB2" y "HER2" se emplean en forma intercambiable en esta solicitud y se refieren a la proteína HER2 humana, descrita por ejemplo en Semba et al (1985) PNAS (USA), 82:6497-6501 y Yamamoto et al (1986) Nature, 319:230-234 (número de acceso Genbank X03363). El término "erbB2" se refiere al gen que codifica el ErbB2 humano y "neu" se refiere al gen que codifica p185neu de rata.

"ErbB3" y "HER3" se refieren al polipéptido de receptor como se divulga, por ejemplo, en US 5183884; US 5480968; Kraus et al (1989) PNAS (USA) 86:9193-9197. Anticuerpos contra ErbB3 son conocidos en el arte (US 5183884; US 5480968; WO 97/35885).

Los términos "ErbB4" y "HER4" en esta solicitud se refieren al polipéptido de receptor como se divulga, por ejemplo, en la Solicitud de Patente EP No. 599,274; Plowman et al., Proc. Natl. Acad. Sci. USA, 90:1746-1750 (1993); y Plowman et al., Nature, 366:473-475 (1993), incluidas sus isoformas, *por ejemplo*, como se divulga en WO 99/19488. Anticuerpos contra HER4 se describen, por ejemplo, en WO 02/18444.

Anticuerpos a receptores ErbB se encuentran disponibles en el comercio de un número de fuentes, incluidas, por ejemplo, Santa Cruz Biotechnology, Inc., California, USA.

Por "ligando ErbB" se da a entender un polipéptido que se une a y/o activa un receptor ErbB. El ligando ErbB puede ser un ligando ErbB humano de secuencia nativa, tal como factor de crecimiento epidérmico (EGF) (Savage et al (1972) J. Biol. Chem., 247:7612-7621); factor de crecimiento transformante alfa (TGF-α) (Marquardt et al (1984) Science 223:1079-1082); anfirregulina, también conocida como schwanoma o factor de crecimiento autocrino de queratinocitos (Shoyab et al (1989) Science 243:1074-1076; Kimura et al (1990) Nature 348:257-260; y Cook et al (1991) Mol. Cell. Biol., 11:2547-2557); betacelulina (Shing et al (1993) Science 259:1604-1607; y Sasada et al (1993) Biochem. Biophys. Res. Commun. 190:1173); factor de crecimiento epidérmico de unión a heparina (HB-EGF) (Higashiyama et al (1991) Science 251:936-939); epirregulina (Toyoda et al (1995) J. Biol. Chem. 270:7495-7500; y Komurasaki et al

(1997) Oncogene 15:2841-2848); una herregulina (véase más adelante); neurregulina-2 (NRG-2) (Carraway et al., Nature, 387:512-516 (1997)); neurregulina-3 (NRG-3) (Zhang et al (1997) Proc. Natl. Acad. Sci., 94:9562-9567); neurregulina-4 (NRG-4) (Harari et al (1999) Oncogene, 18:2681-89) o cripto (CR-1) (Kannan et al (1997) J. Biol. Chem., 272(6):3330-3335). Ligandos ErbB que unen EGFR incluyen EGF, TGF-α, anfirregulina, betacelulina, HB-EGF y epirregulina. Ligandos ErbB que unen ErbB3 incluyen herregulinas. Ligandos ErbB capaces de unir ErbB4 incluyen betacelulina, epirregulina, HB-EGF, NRG-2, NRG-3, NRG-4 y herregulinas. El ligando ErbB puede ser igualmente un ligando ErbB sintético. El ligando sintético puede ser específico para un receptor ErbB particular, o puede reconocer complejos de receptor ErbB particular. Un ejemplo de un ligando sintético es la birregulina de quimera EGF/ herregulina sintética (véase, por ejemplo, Jones et al (1999) FEBS Letters, 447:227-231, que se incorpora por referencia).

"Herregulina" (HRG) se refiere a un polipéptido codificado por el producto génico herregulina como se divulga en US 5641869 o Marchionni et al (1993) Nature 362:312-318. Ejemplos de herregulinas incluyen herregulina-α, herregulina-β1, herregulina-β2 y herregulina-β3 (Holmes et al (1992) Science 256:1205-1210; y US 5641869); factor de diferenciación neu (NDF) (Peles et al (1992) Cell 69: 205-216); actividad inductora de receptor de acetilcolina (ARIA) (Falls et al (1993) Cell 72:801-815); factores de crecimiento de glial (GGFs) (Marchionni et al (1993) Nature, 362:312-318); factor derivado de neurona motora y sensorial (SMDF) (Ho et al (1995) J. Biol. Chem. 270:14523-14532); γ-herregulina (Schaefer et al (1997) Oncogene, 15:1385-1394). El término incluye fragmentos biológicamente activos y variantes de secuencia de aminoácidos de un polipéptido HRG de secuencia nativa, tal como su fragmento de dominio tipo EGF (*por ejemplo*, HRGβ1177-244).

"Hetero-oligómero ErbB" es un oligómero asociado no covalentemente, que comprende por lo menos dos diferentes receptores ERbB. Un "dímero ErbB" es un oligómero asociado no covalentemente,

que comprende dos diferentes receptores ErbB. Tales complejos pueden formarse cuando una célula, que expresa dos o más receptores ErbB, se expone a un ligando ErbB. Los oligómeros ErbB, tales como dímeros ErbB, pueden aislarse por inmunoprecipitación y analizarse por SDS-PAGE como se describe en Sliwkowski et al (1994) J. Biol. Chem., 269(20):14661-14665, por ejemplo. Ejemplos de tales hetero-oligómeros ErbB incluyen complejos EGFR-ErbB2 (también llamado HER1/HER2), ErbB2-ErbB3 (HER2/HER3) y ErbB3-ErbB4 (HER3/HER4). Más aún, el hetero-oligómero ErbB puede comprender dos o más receptores ErbB2 combinados con un receptor ErbB diferente, tal como ErbB3, ErbB4 ó EGFR (ErbB1). Otras proteínas, tales como la subunidad de receptor de citocina (*por ejemplo*, gp130), pueden incluirse en el hetero-oligómero.

Por "activación de ligando de un receptor ErbB" se da a entender transducción de señales (*por ejemplo*, la causada por un dominio cinasa intracelular de un receptor ErbB, que fosforila residuos de tirosina en el receptor ErbB o un polipéptido sustrato), mediada por ligando ErbB que se une a un hetero-oligómero ErbB, que comprende el receptor ErbB de interés. Generalmente, esto involucrará unión de un ligando ErbB a un hetero-oligómero ErbB, el cual activa un dominio cinasa de uno o más de los receptores ErbB en el hetero-oligómero y, por consiguiente, resulta en fosforilación de residuos de tirosina en uno o más de los receptores ErbB y/o fosforilación de residuos de tirosina en polipéptido(s) de sustrato adicional. La activación del receptor ErbB puede cuantificarse utilizando varios ensayos de fosforilación de tirosina.

Un polipéptido "de secuencia nativa" es uno que tiene la misma secuencia de aminoácidos que un polipéptido (*por ejemplo*, receptor ErbB o ligando ErbB) derivado de la naturaleza. Tales polipéptidos de secuencia nativa pueden aislarse de la naturaleza o pueden producirse por medios sintéticos o recombinantes. Por consiguiente, un polipéptido de secuencia nativa puede tener la secuencia de aminoácidos de un polipéptido humano de ocurrencia natural, un polipéptido murino, o un polipéptido de cualquier otra especie mamífera.

El término "variante de secuencia de aminoácidos" se refiere a polipéptidos con secuencias de aminoácidos que difieren en alguna medida de un polipéptido de secuencia nativa. Ordinariamente, variantes de secuencia de aminoácidos poseerán por lo menos alrededor de 70% de identidad de secuencia, con por lo menos un dominio de unión de receptor de un ligando ErbB nativo, o con por lo menos un dominio de unión de ligando de un receptor ErbB nativo, o por lo menos alrededor de 80%, o por lo menos alrededor de 90% homólogo con tales dominios de unión de ligando o receptor. Las variantes de secuencia de aminoácidos poseen sustituciones, eliminaciones, y/o inserciones en ciertas posiciones en la secuencia de aminoácidos de la secuencia de aminoácidos nativa.

"Identidad de secuencia" se define como el porcentaje de residuos en la variante de secuencia de aminoácidos que son idénticos, luego de alinear las secuencias e introducir vacíos, si es necesario, para obtener la máxima identidad de secuencia porcentual. Métodos y programas de computador para la alineación son bien conocidos en el arte. Un programa similar de computadores es "Align 2," creado por Genentech, Inc., el cual se radicó con documentación de usuario en la Oficina de Derechos de Autor de los Estados Unidos, Washington, DC 20559, el 10 de diciembre de 1991.

"Citotoxicidad celular mediada por anticuerpo" y "ADCC" se refieren a una reacción mediada por célula, en la cual células citotóxicas no específicas, que expresan receptores Fc (FcRs) (*por ejemplo*, linfocitos citolíticos naturales (NK), neutrófilos y macrófagos) reconocen un anticuerpo unido en un célula selectiva y, posteriormente, causan lisis de la célula selectiva. Las células primarias para mediar ADCC, linfocitos citolíticos naturales (NK), expresan FcγRIII únicamente, mientras que los monocitos expresan FcγRI, FcγRII y FcγRIII. La expresión de FcR en células hematopoyéticas se resume en la Tabla 3 en página 464 de Ravetch and Kinet, (1991) Annu. Rev. Immunol, 9:457-92. Para evaluar la actividad ADCC de una molécula de interés, puede realizarse un ensayo ADCC *in vitro* (US 55003621; US 5821337). Células efectoras útiles para tales ensayos incluyen células mononucleares de sangre periférica (PBMC) y

linfocitos citolíticos naturales (NK). Alternativa, o adicionalmente, la actividad ADCC de la molécula de interés puede evaluarse *in vivo, por ejemplo*, en un modelo con animales, tal como el divulgado en Clynes et al (1998) PNAS (USA), 95:652-656.

"Fracción de medicamento maitansinoide" significa la subestructura de un conjugado anticuerpo-medicamento, que tiene la estructura de un compuesto de maitansina. La maitansina se aisló por primera vez del arbusto este-africano *Maytenus serrata* (US 3896111). Posteriormente, se descubrió que ciertos microbios también producen maitansinoides, tales como ésteres de maitansinol y C-3 maitansinol (US 4151042). Se han divulgado maitansinol sintético y análogos de maitansinol. Véase Patentes de los Estados Unidos Nos. 4137230; 4248870; 4256746; 4260608; 4265814; 4294757; 4307016; 4308268; 4308269; 4309428; 4313946; 4315929; 4317821; 4322348; 4331598; 4361650; 4364866; 4424219; 4450254; 4362663; y 4371533, y Kawai et al (1984) Chem. Pharm. Bull. 3441-3451), cada una de las cuales se incorporan expresamente por referencia.

Los términos "receptor Fc" o "FcR" se usan para describir un receptor que se une a la región Fc de un anticuerpo, tal como un FcR humano de secuencia nativa. FcR puede unir un anticuerpo IgG (un receptor gamma) e incluye receptores de las subclases FcγRI, FcγRII, y Fcγ RIII, incluidas variantes alélicas y formas alternativamente empalmadas de éstos. Los recptores FcγRII incluyen FcγRIIA (un "receptor activador") y FcγRIIB (un "receptor inhibidor"), que tienen secuencias de aminoácidos similares, que difieren principalmente en sus dominios citoplásmicos. El receptor activador FcγRIIA contiene un motivo de activación basado en tirosina de inmunorreceptor (ITAM) en su dominio citoplásmico. El receptor inhibidor FcγRIIB contiene un motivo de inhibición basado en tirosina de inmunorreceptor (ITIM) en su dominio citoplásmico. (Ver reseña M. en Daëron, Annu. Rev. Immunol., 15:203-234 (1997)). FcRs se reseñan en Ravetch and Kinet, Annu. Rev. Immunol., 9:457-92 (1991); Capel et al (1994) Immunomethods, 4:25-34; y de Haas et al (1995) J. Lab. Clin. Med.,

126:330-41. Otros FcRs, incluidos los por identificar en el futuro, están comprendidos por el término "FcR" en esta solicitud. El término incluye asimismo el receptor neonatal, FcRn, que es responsable de la transferencia de IgGs maternas al feto (Guyer et al (1976) J. Immunol. 117:587, y Kim et al (1994) J. Immunol., 24:249).

"Citotoxicidad dependiente de complemento" o "CDC" se refiere a la capacidad de una molécula de desintegrar, por medio de lisinas, una diana en la presencia de complemento. La vía de activación del complemento se inicia por la unión del primer componente del sistema de complemento (C1q) a una molécula (*por ejemplo*, un anticuerpo) acomplejada con un antígeno afín. Para evaluar la activación del complemento, puede realizarse un ensayo CDC, *por ejemplo*, como se describe en Gazzano-Santoro et al (1996) J. Immunol. Methods, 202:163.

"Anticuerpos nativos" son usualmente glucoproteínas heterotetraméricas de alrededor de 150.000 daltons, compuestas de dos cadenas ligeras (L) idénticas y dos cadenas pesadas (H) idénticas. Cada cadena ligera se conecta a una cadena pesada por un enlace disulfuro covalente, aunque el número de ligamientos disulfuro varía entre las cadenas pesadas de isotipos de inmunoglobulina diferentes. Cada cadena pesada y liviana tiene también puentes disulfuro intra-cadena, regularmente espaciados. Cada cadena pesada tiene en un extremo un dominio variable (VH) seguido por un número de dominios constantes. Cada cadena ligera tiene un dominio variable en un extremo (VL) y un dominio constante en el otro extremo. El dominio constante de la cadena ligera se alinea con el primer dominio constante de la cadena pesada, y el dominio variable de cadena ligera se alinea con el dominio variable de la cadena pesada. Se cree que residuos de aminoácidos particulares forman una interfase entre los dominios variables de cadena ligera y cadena pesada.

El término "variable" se refiere al hecho de que ciertas porciones de los dominios variables difieren extensivamente en secuencia entre anticuerpos y se utilizan en la unión y especificidad de cada anticuerpo particular para su antígeno particular. No obstante, la variabilidad no está

uniformemente distribuida a lo largo y ancho de los dominios variables de los anticuerpos. Ésta se concentra en tres segmentos denominados regiones hipervariables, tanto en los dominios variables de cadena pesada como en los de cadena ligera. Las porciones más altamente conservadas de los dominios variables se llaman las regiones marco (FR). Los dominios variables de cadena nativas pesada y ligera comprenden cada una cuatro FR, que adoptan en gran medida una configuración de hoja β (***"β-sheet configuration"***), conectada por tres regiones hipervariables, que forman lazos que conectan y, en algunos casos, forman parte de la estructura de la hoja β. Las regiones hipervariables en cada cadena se mantienen juntas en estrecha proximidad por las FR y, con las regiones hipervariables de la otra cadena, contribuyen a la formación del sitio de unión de antígeno de anticuerpos (véase Kabat et al (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD). Los dominios constantes no están implicados directamente en unir un anticuerpo a un antígeno, pero exhiben varias funciones efectoras, tales como participación del anticuerpo en la citotoxicidad celular dependiente del anticuerpo (ADCC).

El término "región hipervariable", cuando se usa en esta solicitud, se refiere a los residuos de aminoácidos de un anticuerpo que son responsables de la unión de antígeno. La región hipervariable comprende generalmente residuos de aminoácidos de una "región determinante de complementaridad" o "CDR" (*por ejemplo*, residuos 24-34 (L1), 50-56 (L2) y 89-97 (L3) en el dominio variable de cadena ligera y 31-35 (H1), 50-65 (H2) y 95-102 (H3) en el dominio variable de cadena pesada; Kabat et al., supra) y/o aquellos residuos de un "lazo hipervariable" (*por ejemplo*, residuos 26-32 (L1), 50-52 (L2) y 91-96 (L3) en el dominio variable de cadena ligera y 26-32 (H1), 53-55 (H2) y 96-101 (H3) en el dominio variable de cadena pesada; Chothia y Lesk (1987) J. Mol. Biol. 196: 901-917). Residuos de "región marco" o "FR" son aquellos residuos de dominio variable, distintos de los residuos de la región hipervariable, como se definen en esta solicitud.

La digestión con papaína de anticuerpos produce dos fragmentos idénticos de unión de antígeno, llamados fragmentos "Fab", cada uno con un sitio único de unión de antígeno, y un fragmento "Fc" residual, cuyo nombre refleja su capacidad de cristalizar fácilmente. El tratamiento con pepsina proporciona un fragmento $F(ab')_2$, que tiene dos sitios de unión de antígeno y es aún capaz de enlazar cruzadamente el antígeno.

"Fv" es el fragmento de anticuerpo mínimo, que contiene un sitio completo de unión y reconocimiento de antígeno. Esta región consta de un dímero de un dominio variable de cadena pesada y un dominio variable de cadena ligera, en estrecha asociación no covalente. Es en esta configuración que las tres regiones hipervariables de cada dominio variable interactúan para definir un sitio de unión de antígeno en la superficie del dímero V_H-V_L. Colectivamente, las seis regiones hipervariables confieren especificidad de unión de antígeno al anticuerpo. No obstante, incluso un dominio variable único (o la mitad de un Fv que comprende únicamente tres regiones hipervariables específicas para un antígeno) tiene la capacidad de reconocer y fijar antígeno, si bien con una afinidad más baja que todo el sitio de unión.

El fragmento Fab contiene asimismo el dominio constante de la cadena ligera y el primer dominio constante (CH1) de la cadena pesada. Los fragmentos Fab' difieren de los fragmentos Fab por la adición de unos cuantos residuos en el término carboxi del dominio CH1 de la cadena pesada, incluidas una o más cisteínas de la región flexible del anticuerpo. Fab'-SH es la designación en esta solicitud para Fab', en el cual el residuo(s) de cisteína de los dominios constantes llevan por lo menos un grupo tiol libre. Los fragmentos de anticuerpo $F(ab')_2$ se produjeron originalmente como pares de fragmentos Fab', que tienen cisteínas de unión flexible entre ellos. Se conocen igualmente otros acoplamientos químicos de fragmentos de anticuerpo.

Las "cadenas livianas" de anticuerpos de cualquier especie vertebrada pueden asignarse a uno de dos tipos claramente distintos, llamados kappa

(κ) y lambda (λ), basados en las secuencias de aminoácidos de sus dominios constantes.

"Fv de cadena única" o "scFv" significan fragmentos de anticuerpo de región variable de cadena única, que comprenden los dominios V_H V_L del anticuerpo, en donde estos dominios están presentes en una cadena polipeptídica única. El polipéptido Fv puede comprender además un enlazador de polipéptido entre los dominios V_H y V_L, que permite que el scFv forme la estructura deseada para unión de antígeno (Plückthun en *The Pharmacology of Monoclonal Antibodies,* vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, páginas 269-315(1994). Fragmentos de anticuerpo anti-ErbB se describen en WO 93/16185; US 5571894; US 5587458.

El término "diacuerpos" se refiere a pequeños fragmentos de anticuerpo con dos sitios de unión de antígeno, los cuales fragmentos comprenden un dominio variable de cadena pesada (V_H), conectado un dominio variable de cadena ligera (V_L) en la misma cadena polipeptídica (V_H - V_L). Usando un enlazador, que es demasiado corto para permitir el apareamiento entre los dos dominios en la misma cadena, los dominios son forzados a aparearse con los dominios complementarios de otra cadena y a crear dos sitios de unión de antígeno. Los diacuerpos se describen más completamente en, por ejemplo, EP 404,097; WO 93/11161; y Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448.

Formas "humanizadas" de anticuerpos no humanos (*por ejemplo*, roedor) son anticuerpos quiméricos que contienen una secuencia mínima derivada de inmunoglobulina no humana. En su mayor parte, los anticuerpos humanizados son inmunoglobulinas humanas (anticuerpo destinatario), en las cuales residuos de una región hipervariable del destinatario se reemplazan por residuos de una región hipervariable de una especie no humana (anticuerpo donante) tal como ratón, rata, conejo o primate no humano, que tienen la especificidad, afinidad y capacidad deseadas. En algunos casos, residuos de la región marco (FR) de la inmunoglobulina humana se reemplazan por residuos no humanos

correspondientes. Más aún, los anticuerpos humanizados pueden comprender residuos, que no se encuentran en el anticuerpo destinatario o en el anticuerpo donante. Estas modificaciones se hacen para afinar aún más el desempeño del anticuerpo. En general, el anticuerpo humanizado comprenderá sustancialmente la totalidad de por lo menos uno y, típicamente dos; dominios variables, en los cuales la totalidad o sustancialmente la totalidad de los lazos hipervariables corresponden a los de una inmunoglobulina no humana, y todas o sustancialmente todas las FR son las de una secuencia de inmunoglobulina humana. Opcionalmente, el anticuerpo humanizado comprenderá también por lo menos una porción de una región constante de inmunoglobulina (Fc), típicamente la de una inmunoglobulina humana. Para mayores detalles, véase Jones et al (1986) Nature, 321:522-525; Riechmann et al (1988) Nature 332:323-329; y Presta, (1992) Curr. Op. Struct. Biol., 2:593-596.

Anticuerpos anti-ErbB2 humanizados incluyen huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 y huMAb4D5-8 (HERCEPTIN®, trastuzumab), como se describe en Tabla 3 de US 5821337 incorporada expresamente en esta solicitud por referencia; 520C9 humanizado (WO 93/21319) y anticuerpos 2C4 humanizados.

Un anticuerpo "aislado" es uno que se ha identificado y separado y/o recuperado de un componente de su entorno natural. Componentes contaminantes de su entorno natural son materiales que interferirían los usos diagnósticos o terapéuticos del anticuerpo, y pueden incluir enzimas, hormonas, y otros solutos proteináceos o no proteináceos. El anticuerpo puede purificarse (1) a más del 95% en peso del anticuerpo, según se determine por el método de Lowry, o más del 99% en peso, (2) a un grado suficiente para obtener por lo menos 15 residuos de la secuencia de aminoácidos N-terminal o interna mediante el uso de un secuenciador de vaso giratorio, o (3) a homogeneidad por SDS-PAGE bajo condiciones reductoras o no reductoras usando azul Coomassie o, preferiblemente, tintura de plata. Un anticuerpo aislado incluye el anticuerpo *in situ* en

células recombinantes, puesto que por lo menos un componente del entorno natural del anticuerpo no estará presente. Ordinariamente, sin embargo, el antagonista aislado se preparará en por lo menos un paso de purificación.

Un anticuerpo "que une" un antígeno de interés, *por ejemplo*, antígeno ErbB2, es uno capaz de unir ese antígeno con suficiente afinidad, de modo que el anticuerpo sea útil en seleccionar una célula, que expresa el antígeno. Donde el anticuerpo es uno que une ErbB2, usualmente unirá de manera preferencial ErbB2, por oposición a otros receptores ErbB, y puede ser uno que no reaccione cruzadamente en forma significativa con otras proteínas tales como EGFR, ErbB3 ó ErbB4. En tales incorporaciones, la magnitud de unión del anticuerpo a estas proteínas no-ErbB2 (*por ejemplo*, unión de superficie celular a receptor endógeno) será menor del 10%, determinada por análisis de clasificación celular activada por fluorescencia (FACS) o radioinmunoprecipitación (RIA). A veces, el anticuerpo anti-ErbB2 no reaccionará significativamente de manera cruzada con la proteína neu de rata, *por ejemplo*, como se describe en Schecter et al (1984) Nature 312:513, y Drebin et al (1984) Nature, 312:545-548.

Un anticuerpo que "bloquea" la activación de ligando de un receptor ErbB reduce o previene tal activación, en donde el anticuerpo es capaz de bloquear la activación de ligando del receptor ErbB de manera sustancialmente más efectiva que el anticuerpo monoclonal 4D5, *por ejemplo*, casi tan efectivamente como los anticuerpos monoclonales 7F3 ó 2C4 o sus fragmentos Fab. Por ejemplo, el anticuerpo que bloquea la activación de ligando de un receptor ErbB puede ser uno que es alrededor de 50-100% más efectivo que 4D5 en bloquear la formación de un hetero-oligómero ErbB. El bloqueo de la activación de ligando de un receptor ErbB puede ocurrir por cualquier medio, *por ejemplo*, interfiriendo: la unión de ligando a un receptor ErbB, la formación de complejo ErbB, la actividad de tirosina cinasa de un receptor ErbB en un complejo ErbB y/o la fosforilación de residuo(s) de tirosina cinasa en o por un receptor ErbB.

Un anticuerpo que tiene una "característica biológica" de un anticuerpo designado, tal como el anticuerpo monoclonal designado 2C4

(Omnitarg, Genentech, Inc.), es uno que posee una o más de las características biológicas de ese anticuerpo, que lo distinguen de otros anticuerpos que unen el mismo antígeno (*por ejemplo*, ErbB2). Por ejemplo, un anticuerpo con una característica biológica de 2C4 puede bloquear la activación HRG de un hetero-oligómero ErbB, que comprende ErbB2 y ErbB3, ErbB1 ó ErbB4; bloquear EGF, TGF-α, HB-EGF, activación de epirregulina y/o anfirregulina de un receptor ErbB, que comprende EGFR y ErbB2; bloquear EGF, TGF-α y/o activación, mediada por HRG, de MAPK; y/o unir el mismo epítopo en el dominio extracelular de ErbB2 como el ligado por 2C4 (*por ejemplo*, que bloquea la unión del anticuerpo monoclonal 2C4 a ErbB2).

A menos que se indique lo contrario, la expresión "anticuerpo monoclonal 2C4" se refiere a un anticuerpo que tiene residuos de unión de antígeno, o derivado, del anticuerpo 2C4 murino de los Ejemplos que siguen. Por ejemplo, el anticuerpo monoclonal 2C4 puede ser un anticuerpo monoclonal 2C4 murino o su variante, tal como un anticuerpo 2C4 humanizado, que posee residuos de aminoácidos de unión de antígeno de anticuerpo monoclonal 2C4 murino (WO 01/00245). A menos que se indique lo contrario, la expresión "rhuMAb 2C4", cuando se emplea en esta solicitud, se refiere a un anticuerpo que comprende las secuencias variables ligeras (VL) y variables pesadas (VH) de SEQ ID Nos. 3 y 4, respectivamente, fusionadas a secuencias de región constante de IgG1 (no alotipo A) ligera y pesada humana, expresada opcionalmente por una célula de Ovario de Hámster de la China (CHO) (WO 01/00245).

A menos que se indique lo contrario, el término "anticuerpo monoclonal 4D5" se refiere a un anticuerpo que tiene residuos de unión de antígeno, o derivado, del anticuerpo 4D5 murino (ATCC CRL 10463). Por ejemplo, el anticuerpo monoclonal 4D5 puede ser un anticuerpo monoclonal murino 4D5 o su variante, tal como un 4D5 humanizado, que posee residuos de unión de antígeno de anticuerpo monoclonal murino 4D5. Anticuerpos 4D5 humanizados ejemplares incluyen huMAb4D5-1,

huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 y huMAb4D5-8 (HERCEPTIN®) como en US 5821337.

Un "agente inhibidor de crecimiento" se refiere a un compuesto o composición que inhibe el crecimiento de una célula, *por ejemplo* una célula cancerosa, que expresa ErbB ya sea *in vitro* o *in vivo*. Por consiguiente, el agente inhibidor de crecimiento puede ser uno que reduzca significativamente el porcentaje de células, que expresan ErbB en la fase S. Ejemplos de agentes inhibidores de crecimiento incluyen agentes que bloquean la progresión del ciclo celular (en un sitio distinto de la fase S), tales como agentes que inducen la detención G1 y la detención de fase M (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). Ejemplos de anticuerpos "inhibidores de crecimiento" son aquellos que se unen a ErbB2 e inhiben el crecimiento de células cancerosas, que sobreexpresan ErbB2. Anticuerpos anti-ErbB2 inhibidores de crecimiento pueden inhibir el crecimiento de células tumorales de mama SK-BR-3 en cultivo celular en más del 20%, o más del 50% (*por ejemplo*, desde alrededor de 50% a alrededor de 100%) con una concentración de anticuerpo de alrededor de 0.5 a 30 µg/ml, donde la inhibición del crecimiento se determina seis días después de la exposición de las células SK-BR-3 al anticuerpo (US 5677171).

Un anticuerpo que "induce muerte celular" es uno que hace que una célula viable se vuelva una célula no viable. Generalmente la célula es una que expresa el receptor ErbB2, especialmente donde la célula sobreexpresa el receptor ErbB2. La célula puede ser una célula cancerosa, *por ejemplo*, una célula de mama, ovario, estómago, endometrio, glándula salival, pulmón, riñón, colon, tiroides, páncreas o vejiga. *In vitro*, la célula puede ser una célula SK-BR-3, BT474, Calu 3, MDA- MB-453, MDA-MB-361 ó SKOV3. La muerte celular *in vitro* puede determinarse en la ausencia de complemento y células efectoras inmunitarias para distinguir la muerte celular inducida por citotoxicidad celular dependiente de anticuerpo

(ADCC), o citotoxicidad dependiente de complemento (CDC). Por consiguiente, el ensayo para determinar la muerte celular puede llevarse a cabo usando suero inactivado por calor (*es decir*, en la ausencia de complemento) y en la ausencia de células efectoras inmunitarias. Para determinar si el anticuerpo es capaz de inducir muerte celular, puede evaluarse la pérdida de integridad de la membrana, evaluada por la captación de yoduro de propidio (PI), colorante *trypan blue* (véase Moore et al (1995) Cytotechnology, 17:1-11) o 7AAD, en relación con células no tratadas. Anticuerpos que inducen muerte celular son aquellos que inducen la captación de PI en el ensayo de captación de PI en células BT474 (vér más adelante).

Un anticuerpo que "induce apoptosis" es uno que induce una muerte celular programada, como se determina por unión de anexina V, fragmentación de ADN, encogimiento celular, dilatación del retículo endoplasmático, fragmentación celular, y/o formación de vesículas de membrana (llamadas cuerpos apoptóticos). Usualmente la célula es una que sobreexpresa el receptor ErbB2, incluida una célula tumoral, *por ejemplo*, una célula de mama, ovario, estómago, endometrio, glándula salival, pulmón, riñon, colon, tiroides, páncreas o vejiga. *In vitro*, la célula puede ser una célula SK-BR-3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 ó SKOV3.

Los términos "tratar" o "tratamiento" se refieren al tratamiento terapéutico y profiláctico, o a medidas preventivas, donde el objeto es prevenir o desacelerar (disminuir) un cambio o trastorno fisiológico indeseado, tal como la evolución o difusión de cáncer. Para efectos de esta invención, resultados clínicos deseados o benéficos incluyen, pero no se limitan a, alivio de síntomas, disminución de la magnitud de la enfermedad, estado estabilizado (*es decir*, no empeoramiento) de la enfermedad, retraso o desaceleramiento de la evolución de la enfermedad, mejoría o alivio del estado patológico, y remisión (parcial o total), sea detectable o indetectable. "Tratamiento" puede significar igualmente prolongar la supervivencia, comparada con la supervivencia esperada, si no se recibiera tratamiento.

Las personas necesitadas de tratamiento incluyen aquellas que ya sufren de la enfermedad o trastorno, así como aquellas propensas a padecer la enfermedad o trastorno, o aquellas en las cuales la enfermedad o trastorno se va a prevenir.

Un "trastorno" es cualquier afección que se beneficiaría del tratamiento de la presente invención. Esto incluye trastornos crónicos y agudos o enfermedades, incluidas aquellas condiciones patológicas que predisponen al mamífero al trastorno en cuestión. Ejemplos no limitantes de trastornos que pueden tratarse en esta solicitud incluyen tumores benignos y malignos; leucemia y neolplasias malignas linfoides, en particular cáncer de mama, ovarios, estómago, endometrio, glándula salival, pulmón, riñón, colon, tiroides, páncreas, próstata o vejiga; trastornos neuronales, gliales, astrocíticos, hipotalámicos y otros trastornos glandulares, macrofágicos, epiteliales, estromales y blastocélicos; y trastornos inflamatorios, angiogénicos e inmunológicos. Un trastorno ejemplar a tratarse de acuerdo con la presente invención es un tumor maligno sólido.

El término "cantidad terapéuticamente efectiva" se refiere a una cantidad de un medicamento, efectiva para tratar una enfermedad o desorden en un mamífero. En el caso de cáncer, la cantidad terapéuticamente efectiva del medicamento puede: (i) reducir el número de células cancerosas; (ii) reducir el tamaño del tumor; (iii) inhibir, retardar, desacelerar en alguna medida y, preferiblemente, detener y la infiltración de células cancerosas en órganos periféricos; (iv) inhibir (*es decir*, desacelerar en alguna medida y, preferiblemente, detener) la metástasis del tumor; (v) inhibir el crecimiento del tumor; y/o (vi) aliviar en alguna medida uno o más de los síntomas asociados con el cáncer. En la medida en que el medicamento puede prevenir el crecimiento y/o eliminar células cancerosas existentes, éste puede ser citostático y/o citotóxico. En modelos con animales, la eficacia puede evaluarse mediante mediciones físicas del tumor durante el transcurso del tiempo, luego de la administración del ADC, y determinando la remisión parcial y completa del tumor. Para terapia de

cáncer, la eficacia puede medirse, por ejemplo, evaluando el tiempo con respecto a la evolución de la enfermedad (TTP) y/o determinando la velocidad de respuesta (RR).

El término "biodisponibilidad" se refiere a la disponibilidad sistémica (*es decir*, niveles en sangre/plasma) de una cantidad dada de medicamento administrado a un paciente. Biodisponibilidad es un término absoluto que indica la medición tanto del tiempo (velocidad) como de la cantidad (cuantía) de medicamento que llega a la circulación general de una forma farmacéutica administrada.

Los términos "cáncer" y "canceroso" se refieren o describen la condición fisiológica en mamíferos, que se caracteriza típicamente por un crecimiento celular descontrolado. Un "tumor" comprende una o más células cancerosas. Ejemplos de cáncer incluyen, pero no se limitan a, carcinoma, linfoma, blastoma, sarcoma, y leucemia o neoplasias malignas linfoides. Ejemplos más particulares de tales neoplasias malignas incluyen carcinoma epidermoide (***"squamous cell cancer"***) (*por ejemplo*, carcinoma epidermoide epitelial), cáncer de pulmón, incluidos cáncer de pulmón microcítico, cáncer de pulmón amicrocítico ("NSCLC"), adenocarcinoma del pulmón y carcinoma epidermoide del pulmón, cáncer del peritoneo, cáncer hepatocelular, cáncer gástrico o del estómago, incluido cáncer gastrointestinal, tumor estromal gastrointestinal (GIST), cáncer pancreático, glioblastoma, cáncer cervical, cáncer de ovarios, cáncer del hígado, cáncer de la vejiga, hepatoma, cáncer de mama, cáncer de colon, cáncer rectal, cáncer colorrectal, cáncer del endometrio o carcinoma uterino, carcinoma de glandula salival, cáncer de riñón o renal, cáncer de próstata, cáncer de la vulva, cáncer de la tiroides, carcinoma hepático, carcinoma anal, carcinoma del pene, así como cáncer de cabeza y cuello.

Un "cáncer que expresa ErbB" es uno que comprende células con proteína ErbB presente en su superficie celular. Un cáncer "que expresa ErbB2" es uno que produce niveles suficientes de ErbB 2 en la superficie de sus células, de modo que un anticuerpo anti-ErbB2 puede unirse a ellas y tener un efecto terapéutico con respecto al cáncer.

Un cáncer que "sobreexpresa" un receptor, *por ejemplo* un receptor ErbB, es uno que tiene niveles significativamente más altos del receptor, tal como ErbB2, en su superficie celular, comparado con una célula no cancerosa del mismo tipo de tejido. Tal sobreexpresión puede ser causada por amplificación génica o por transcripción o traducción incrementada. La sobreexpresión de receptor ErbB puede determinarse en un análisis diagnóstico o prognóstico evaluando niveles crecientes de la proteína ErbB presente en la superficie de una célula (*por ejemplo*, mediante un análisis de inmunohistoquímica; IHC). Alternativa, o adicionalmente, se pueden medir niveles de ácido nucleico codificante de ErbB en la célula, *por ejemplo*, mediante hibridación *in situ* fluorescente (FISH; véase WO 98/45479), técnicas southern blotting, o reacción en cadena de polimerasa (PCR), tal como PCR cuantitativa en tiempo real (RT-PCR). La sobreexpresión del ligando ErbB puede determinarse diagnósticamente evaluando niveles del ligando (o ácido nucleico que lo codifica) en el paciente, *por ejemplo*, en una biopsia del tumor o mediante diferentes análisis diagnósticos tales como los análisis IHC, FISH, southern blotting, PCR o *in vivo* descritos anteriormente. Se puede estudiar también la sobreexpresión del receptor ErbB midiendo el antígeno circulante (*por ejemplo*, dominio extracelular ErbB) en un fluido biológico tal como suero (véase, *por ejemplo*, US 4933294; WO 91/05264; US 5401638; y Sias et al (1990) J. Immunol. Methods 132: 73-80). Aparte de los análisis anteriores, se encuentran a disposición del médico profesional varios otros análisis *in vivo*. Por ejemplo, se pueden exponer células dentro del organismo del paciente a un anticuerpo, que se marca opcionalmente con un marcador detectable, *por ejemplo*, un isótopo radioactivo, y puede evaluarse la unión del anticuerpo a células en el paciente, *por ejemplo*, mediante barrido externo para detectar radioactividad, o analizando una biopsia tomada de un paciente previamente expuesto al anticuerpo.

Los tumores que sobreexpresan HER2 se califican con puntajes inmunohistoquímicos correspondientes al número de copias de moléculas HER2 expresadas por célula, y pueden determinarse bioquímicamente: 0 =

0-10.000 copias/célula, 1+ = por lo menos alrededor de 200.000 copias/célula, 2+ = por lo menos alrededor de 500.000 copias/célula, 3+ = alrededor de 1-2 x 10^5 copias/célula. La sobreexpresión de HER2 en el nivel 3+, que lleva a una activación independiente de ligando de la tirosina cinasa (Hudziak et al (1987) Proc. Natl. Acad. Sci. USA 84:7159-7163), ocurre en aproximadamente 30% de las neoplasias malignas de mama y, en estos pacientes, la supervivencia libre de recaída y la supervivencia general se encuentran disminuídas (Slamon et al (1989) Science 244:707-712; Slamon et al (1987) Science, 235:177-182). Inversamente, un cáncer que "no se caracteriza por sobreexpresión del receptor ErbB2" es uno que, en un análisis diagnóstico, no expresa niveles más elevados que los normales de receptor ErbB2, comparado con una célula no cancerosa del mismo tipo de tejido. El anticuerpo anti-HER2 monoclonal murino inhibe el crecimiento de líneas celulares de cáncer de mama, que sobreexpresan HER2 en el nivel 2+ y 3+ (1-2 x 10^6 receptores HER2 por célula), pero carece de actividad en células que expresan niveles más bajos de HER2 (Lewis et al (1993) Cancer Immunol. Immunother. 37:255-263). Basados en esta observación, se humanizó el anticuerpo 4D5 (huMAb4D5-8, rhuMAb HER2, US 5821337; Carter et al (1992) Proc. Natl. Acad. Sci. USA 89: 4285-4289) y se probó en pacientes con cáncer de mama, cuyos tumores sobreexpresaban HER2, pero que habían evolucionado luego de quimioterpaia convencional (Cobleigh et al (1999) J. Clin. Oncol. 17: 2639-2648). La mayoría de los tumores de pacientes en este estudio clínico expresaron HER2 en el nivel 3+, aunque una fracción fue 2+.

Un cáncer "independiente de hormona" es uno en el cual su proliferación no es dependiente de la presencia de una hormona, que se une a un receptor expresado por células en el cáncer. Tales neoplasias malignas no experimentan regresión clínica con la administración de estrategias farmacológicas o quirúrgicas que reducen la concentración de hormona en o cerca del tumor. Ejemplos de neoplasias malignas independientes de hormona incluyen cáncer de próstata independiente de andrógeno, cáncer mama independiente de estrógeno, cáncer del endometrio y cáncer de los

ovarios. Tales neoplasias malignas pueden comenzar como tumores dependientes de hormona y evolucionar, de una fase sensible a hormona, a un tumor resistente a la hormona, luego de la terapia anti-hormonal.

El término "agente citotóxico", tal como se usa en esta solicitud, se refiere a una sustancia que inhibe o previene la función de las células y/o causa destrucción de las células. El término tiene como finalidad incluir isótopos radioactivos (*por ejemplo*, ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P e isótopos radioactivos de Lu), agentes quimioterapéuticos, y toxinas tales como toxinas de molécula pequeña o toxinas enzimáticamente activas de origen bacteriano, fúngico, vegetal o animal, o sus fragmentos.

Un "agente quimioterapéutico" es un compuesto químico útil en el tratamiento de cáncer. Ejemplos de agentes quimioterapéuticos incluyen Erlotinib (TARCEVA®, Genentech/OSI Pharm.), Bortezomib (VELCADE®, Millenium Pharm.), Fulvestrant (FASLODEX®, Astrazeneca), Sutent (SU11248, Pfizer), Letrozol (FEMARA®, Novartis), Imatinib mesilato (GLEEVEC®, Novartis), PTK787/ZK 222584 (Novartis), Oxaliplatin (Eloxatin®, Sanofi), 5-FU (5-fluorouracilo), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (GSK572016, GlaxoSmithKline), Lonafarnib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs.), y Gefitinib (IRESSA®, Astrazeneca), AG1478, AG1571 (SU 5271; Sugen), agentes alquilantes tales como tiotepa y CYTOXAN® ciclosfosfamida; alquilsulfonatos tales como busulfán, improsulfán y piposulfán; aziridinas tales como benzodopa, carbocuona, meturedopa, y uredopa; etileniminas y metilamelaminas incluidas altretamina, trietilenmelamina, trietilenfosforamida, trietilentiofosforamida y trimetilomelamina; acetogeninas (especialmente bulatacina y bulatacinona); una camptotecina (incluido el análogo sintético topotecán); briostatina; calistatina; CC-1065 (incluidos sus análogos sintéticos adozelesina, carzelesina y bizelesina); criptoficinas (particularmente criptoficina 1 y criptoficina 8); dolastatina; duocarmicina (incluidos los análogos sintéticos, KW-2189 y CB1-TM1); eleuterobina; pancratistatina; una sarcodictina; espongistatina; mostazas nitrogenadas tales como

clorambucil, clornafazina, colofosfamida, estramustina, ifosfamida, mecloretamina, clorhidrato de óxido de mecloretamina, melfalán, novembichin, phenesterine, prednimustina, trofosfamida, mostaza de uracilo; nitrosureas tales como carmustina, clorozotocina, fotemustina, lomustina, nimustina, y ranimnustina; antibióticos tales como los antibióticos enediyne (*por ejemplo*, caliqueamicina, especialmente caliqueamicina gamma1I y caliqueamicina omega1I (Agnew, Chem Intl. Ed. Engl., (1994) 33: 183-186); dinemicina, incluida dinemicina A; bisfosfonatos, tales como clodronato; una esperamicina; así como cromóforo de neocarzinostatina y cromóforos antibióticos relacionados cromoproteína enediyne), aclacinomisinas, actinomicina, autramicina, azaserina, bleomicinas, cactinomicina, carabicina, carminomicina, carzinofilina, cromomicinas, dactinomicina, daunorubicina, detorubicina, 6-diazo-5-oxo-L-norleucina, ADRIAMYCIN® doxorubicina (incluidas morfolino-doxorubicina, cianomorfolino-doxorubicina, 2-pirrolino-doxorubicina y deoxidoxorubicina), epirubicina, esorubicina, idarubicina, marcelomicina, mitomicinas tales como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatina, zorubicina; anti-metabolitos tales como metotrexato y 5-fluorouracilo (5-FU); análogos de ácido fólico tales como denopterina, metotrexato, pteropterina, trimetrexato; análogos de purina tales como fludarabina, 6-mercaptopurina, tiamiprina, tioguanina; análogos de pirimidina tales como ancitabina, azacitidina, 6-azauridina, carmofur, citarabina, dideoxiuridina, doxifluridina, enocitabina, floxuridina; andrógenos tales como calusterona, dromostanolona propionato, epitiostanol, mepitiostano, testolactona; anti-adrenales tales como aminoglutetimida, mitotano, trilostano; reabastecedor de ácido fólico tal como ácido frolínico; aceglatona; glucósido de aldofosfamida; ácido aminolevulínico; eniluracilo; amsacrina; bestrabucil; bisantreno; edatraxato; defofamine; demecolcine; diaziquone; elfornithine; acetato de eliptinio; una epotilona; etoglucid; nitrato de galio; hidroxiurea; lentinan; lonidainine;

maitansinoides tales como maitansina y ansamitocinas; mitoguazona; mitoxantrona; mopidanmol; nitraerine; pentostatina; phenamet; pirarubicina; losoxantrona; ácido podofilínico; 2-etilhidrazida; procarbazina; complejo polisacárido PSK® (JHS Natural Products, Eugene, OR); razoxano; rizoxina; sizofirano; espirogermanio; ácido tenuazónico; triazicuona; 2,2',2"- triclorotrietilamina; tricotecenos (especialmente T-2 toxina, verracurin A, roridin A y anguidine); uretano; vindesina; dacarbazina; mannomustina; mitobrónitol; mitolactol; pipobroman; gacytosine; arabinósido ("Ara-C"); ciclofosfamida; tiotepa; taxoides, *por ejemplo*, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ formulación de nanopartículas diseñadas con albúmina, libres de cremóforo, de paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), y TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, Francia); clorambucil; GEMZAR® gemcitabina; 6-tioguanina; mercaptopurina; metotrexato; análogos de platino tales como cisplatino y carboplatino; vinblastina; platino; etopósido (VP-16); ifosfamida; mitoxantrona; vincristina; NAVELBINE® vinorelbina; novantrona; tenipósido; edatrexato; daunomicina; aminopterina; xeloda; ibandronato; CPT-11; inhibidor de toposiomerasa RFS 2000; difluorometilornitina (DMFO); retinoides tales como ácido retinoico; capecitabina; y sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores.

También se encuentran incluidos en esta definición de "agente quimioterapéutico": (i) agentes anti-hormonales que actúan para regular o inhibir la acción hormonal en tumores tales como anti-estrógenos y moduladores selectivos de receptor de estrógeno (SERM), incluidos, por ejemplo, tamoxifeno (incluido NOLVADEX® tamoxifeno), raloxifeno, droloxifeno, 4-hidroxitamoxifeno, trioxifeno, keoxifeno, LY117018, onapristone, y FARESTON toremifeno; (ii) inhibidores de aromatasa que inhiben la enzima aromatasa, la cual regula la producción de estrógeno en las glándulas suprarrenales, tales como, por ejemplo, 4(5)-imidazoles, aminoglutetimida, MEGASE® acetato de megestrol, AROMASIN®

exemestano, formestane, fadrozol, RIVISOR® vorozol, FEMARA® letrozol, y ARIMIDEX® anastrozol; (iii) anti-andrógenos tales como flutamida, nilutamida, bicalutamida, leuprolida, y goserelina; así como troxacitabina (un análogo de citosina nucleósido 1,3-dioxolano); (iv) inhibidores de aromatasa; (v) inhibidores de proteína cinasa; (vi) inhibidores de cinasa lipídica; (vii) oligonucleótidos complementarios, particularmente aquellos que inhiben la expresión de genes en las vías de señalización, implicados en proliferación celular aberrante, tales como, por ejemplo, PKC-alfa, Ralf y H-Ras; (viii) ribozimas tales como el inhibidor de expresión VEGF (*por ejemplo*, ribozima ANGIOZYME®) y un inhibidor de expresión HER2; (ix) vacunas tales como vacunas de terapia génica, por ejemplo, vacuna ALLOVECTIN®, vacuna LEUVECTIN®, y vacuna VAXID®; PROLEUKIN® rIL-2; LURTOTECAN® inhibidor de topoisomerasa 1; ABARELIX® rmRH; y (xi) y sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores.

Inhibidores de proteína cinasa incluyen inhibidores de tirosina cinasa, los cuales inhiben en cierta medida la actividad tirosina cinasa de una tirosina cinasa tal como un receptor ErbB. Ejemplos de inhibidores de tirosina cinasa incluyen medicamentos con selectividad para EGFR tales como: (i) anticuerpos que se unen a EGFR, incluidos MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (véase, US 4943533, Mendelsohn *et al.*) y sus variantes, tal como 225 quimerizado (C225 ó Cetuximab; ERBITUX®, Imclone) y 225 humano reconfigurado ($H_2$25) (WO 96/40210, Imclone Systems Inc.); anticuerpos que unen EGFR mutante tipo II (US 5212290); anticuerpos quiméricos y humanizados que unen EGFR (US 5891996); y anticuerpos humanos que unen EGFR, tales como ABX-EGF (WO 98/50433); (ii) anticuerpo anti-EGFR conjugado con un agente citotóxico (EP 659439A2); y moléculas pequeñas que se unen a EGFR incluidas ZD1839 o Gefitinib (IRESSA™; Astra Zeneca), Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI) y AG1478, AG1571 (SU 5271; Sugen), quinazolinas tales como PD 153035,4-(3-

cloroanilino)quinazolina, piridopirimidinas, pirimidopirimidinas, pirrolopirimidinas, tales como CGP 59326, CGP 60261 y CGP 62706, y pirazolopirimidinas, A-(fenilamino)-7H-pirrolo[2,3-d]pirimidinas, curcumin (diferuloil metano, 4,5-bis (4-fluoroanilino)ftalimida), trifostinas que contienen fracciones de nitrotiofeno; PD-0183805 (Warner-Lambert); moléculas complementarias (*por ejemplo*, aquellas que se unen a ácido nucleico codificante de ErbB); quinoxalinas (US 5804396); trifostinas (US 5804396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); inhibidores de pan-ErbB tales como CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesilato (Gleevac; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxanib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-IC11 (Imclone); o como se describe en: US 5804396; WO 99/09016 (American Cyanamid); WO 98/43960 (American Cyanamid); WO 97/38983 (Warner Lambert); WO 99/06378 (Warner Lambert); WO 99/06396 (Warner Lambert); WO 96/30347 (Pfizer, Inc); WO 96/33978 (Zeneca); WO 96/3397 (Zeneca); y WO 96/33980 (Zeneca).

Un "agente anti-angiogénico" se refiere a un compuesto que bloquea, o interfiere en algún grado, el desarrollo de vasos sanguíneos. Por ejemplo, el factor anti-angiogénico puede ser una molécula pequeña o anticuerpo que se une a un factor de crecimiento o receptor de factor de crecimiento, involucrado en promover angiogénesis. Un agente anti-angiogénico ejemplar es un anticuerpo que se une al Factor de Crecimiento Endotelial Vascular (VEGF), tal como bevacizumab (AVASTIN®, Genentech).

El término "citocina" es un término genérico para proteínas liberadas por una población de células que actúan sobre otra célula como mediadores intercelulares. Ejemplos de tales citocinas son linfocinas, monocinas, y hormonas polipeptídicas tradicionales. Incluidas entre las citocinas se encuentran somatotropina tal como somatotropina humana, somatotropina humana N-metionilo, y somatotropina bobina; hormona paratiroide; tiroxina; insulina; proinsulina; relaxina; prorrelaxina; hormonas de glucoproteína tales como folitropina (FSH), tirotropina (TSH), y lutropina

(LH); somatotropina hepática; factor de crecimiento de fibroblastos; prolactina; lactógeno placentario; factor de necrosis tumoral α y β; sustancia inhibidora de Müller; péptido asociado con gonadotropina de ratón; inhibina; activina; factor de crecimiento endotelial vascular; integrina; trombopoyetina (TPO); factores de crecimiento neurales tales como NGF-β; factor de crecimiento plaquetario; factores de crecimiento tansformantes (TGF) tales como TGF-α y TGF-β; factor de crecimiento insulinoide tipo I y II; eritropoyetina (EPO); factores osteoinductores; interferones tales como interferón α, β, y γ; factores estimulantes de colonias (CSFs) stales como CSF de macrófago (M-CSF); CSF de granulocito-macrófago (GM-CSF); y CSF de granulocito (G-CSF); interleucinas (ILs) tales como IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; un factor de necrosis tumoral tal como TNF-α ó TNF-β; y otros factores polipeptídicos incluido LIF y ligando de kit (KL). Tal como se emplea en esta solicitud, el término citocina incluye proteínas de fuentes naturales o de cultivo de células recombinantes y equivalentes biológicamente activos de las citocinas de secuencia nativa.

El término "profármaco", tal como se emplea en esta solicitud, se refiere a un precursor o forma derivada de una sustancia farmacéuticamente activa, que es menos citotóxica a las células tumorales, comparada con el medicamento original, y que es capaz de activarse enzimáticamente o convertirse en la forma original más activa. Véase, *por ejemplo*, Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) y Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al* (ed.), pp. 247-267, Humana Press (1985). Los profármacos de esta invención incluyen, pero no se limitan a, profármacos con contenido de fosfato, profármacos con contenido de tiofosfato, profármacos con contenido de sulfato, profármacos con contenido de péptido, profármacos modificados con D-aminoácidos, profármacos glucosilados, profármacos con contenido de β-lactama, profármacos con contenido de fenoxiacetamida opcionalmente sustituida, o profármacos con contenido de fenilacetamida

opcionalmente sustituida, profármacos de 5-fluorocitosina y otros profármacos de 5-fluorouridina, que pueden convertirse en el medicamento libre citotóxico más activo. Ejemplos de medicamentos citotóxicos, que pueden derivarse en una forma profármaco para uso en esta invención, incluyen, pero no se limitan a, aquellos agentes quimioterapéuticos ya descritos.

Un "liposoma" es una pequeña vesícula compuesta de varios tipos de lípidos, fosfolípidos y/o surfactante, que es útil para dispensar un medicamento (tal como los anticuerpos anti-ErbB2 divulgados en esta solicitud y, opcionalmente, un agente quimioterapéutico) a un mamífero. Los componentes del liposoma se disponen comúnmente en una formación bicapa, similar a la disposición de los lípidos de las membranas biológicas.

El término "prospecto del envase" se emplea para referirse a instrucciones usualmente incluidas en los envases comerciales de productos terapéuticos, que contienen información acerca de las indicaciones, uso, posología, administración, contraindicaciones y/o advertencias concernientes al empleo de tales productos terapéuticos.

Un "cardioprotector" es un compuesto o composición que previene o reduce la disfunción del miocardio (*es decir*, cardiomiopatía y deficiencia cardiaca congestiva), asociada con la administración de un medicamento, tal como un antibiótico antraciclina y/o un anticuerpo anti-ErbB2, a un paciente. El cardioprotector puede, por ejemplo, bloquear o reducir un efecto cardiotóxico, mediado por radicales libres, y/o prevenir o reducir la lesión por estrés oxidativo. Ejemplos de cardioprotectores abarcados por la presente definición incluyen el agente quelante de hierro dexrazoxano (ICRF-187) (Seifert et al., *The Annals of Pharmacotherapy*, 28:1063-1072 (1994)); un agente reductor de lípido y/o anti-oxidante tal como probucol (Singal et al., *J. MoL Cell Cardiol*, 27:1055-1063 (1995)); amifostina (éster de aminotiol 2-[(3-aminopropil)amino]etanotiol-dihidrógeno fosfato, llamado también WR-2721, y su forma de captación celular desfosforilada llamada WR-1065) y ácido S-3-(3-metilaminopropilamino)propilfosforotiónico (WR-151327), véase Green et

al (1994) Cancer Research, 54:738-741; digoxina (Bristow, M.R. ed. (1980) *Drug-Induced Heart Disease*. New York: Elsevier 191-215); beta-bloqueadores tales como metoprolol (Hjalmarson et al (1994) Drugs 47: Suppl 4:31-9; y Shaddy et al (1995) Am. Heart J., 129:197-9); vitamina E; ácido ascórbico (vitamina C); barredores de radicales libres tales como ácido oleanolico, ácido ursólico y N-acetilcisteína (NAC); compuestos atrapadores de espín tales como alfa-fenil-*terc*-butil nitrona (PBN); (Paracchini et al (1993) Anticancer Res., 13:1607-1612); compuestos selenoorgánicos tales como P251 (Elbesen); y similares.

Una molécula de ácido nucleico "aislada" es una molécula de ácido nucleico que se identifica y separa de por lo menos una molécula de ácido nucleico contaminante, con la cual ordinariamente se asocia en la fuente natural del ácido nucleico del anticuerpo. Una molécula de ácido nucleico aislada difiere de la forma o escenario, en el cual se encuentra en la naturaleza. Por lo tanto, moléculas de ácido nucleico aisladas se distinguen de la molécula de ácido nucleico tal como existe en células naturales. No obstante, una molécula de ácido nucleico aislada incluye una molécula de ácido nucleico contenida en células, que ordinariamente expresan el anticuerpo donde, por ejemplo, la molécula de ácido nucleico está en una ubicación cromosómica diferente de la de células naturales.

"Alquilo" es C_1-C_{18} hidrocarburo que contiene átomos de carbono normal, secundario, terciario o cíclico. Ejemplos de radicales alquilo incluyen fracciones C_1-C_8 hidrocarburo tales como, pero no limitadas a: metilo (Me, $-CH_3$), etilo (Et, $-CH_2CH_3$), 1-propilo (n-Pr, n-propilo, $-CH_2CH_2CH_3$), 2-propilo (i-Pr, i-propilo, $-CH(CH_3)_2$), 1-butilo (n-Bu, n-butilo, $-CH_2CH_2CH_2CH_3$), 2-metil-1-propilo (i-Bu, i-butilo, $-CH_2CH(CH_3)_2$), 2-butilo (s-Bu, s-butilo, $-CH(CH_3)CH_2CH_3$), 2-metil-2-propilo (t-Bu, t-butilo, $-C(CH_3)_3$), 1-pentilo (n-pentilo, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentilo ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentilo ($-CH(CH_2CH_3)_2$), 2-metil-2-butilo ($-C(CH_3)_2CH_2CH_3$), 3-metil-2-butilo ($-CH(CH_3)CH(CH_3)_2$), 3-metil-1-butilo ($-CH_2CH_2CH(CH_3)_2$), 2- metil-1-butilo ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexilo ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-

hexilo (-$CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexilo (-$CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-metil-2-pentilo (-$C(CH_3)_2CH_2CH_2CH_3$), 3-metil-2-pentilo (-$CH(CH_3)CH(CH_3)CH_2CH_3$), 4-metil-2-pentilo (-$CH(CH_3)CH_2CH(CH_3)_2$), 3-metil-3-pentilo (-$C(CH_3)(CH_2CH_3)_2$), 2-metil-3-pentilo (-$CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimetil-2-butilo (-$C(CH_3)_2CH(CH_3)_2$), 3,3-dimetil-2-butilo ($CH(CH_3)C(CH_3)_3$.

"Alquenilo" es C_2-C_{18} hidrocarburo que contiene átomos de carbono normal, secundario, terciario o cíclico con por lo menos un sitio de insaturación, *es decir*, un enlace doble sp^2 carbono-carbono. Ejemplos de radicales alquenilo incluyen fracciones C_2-C_8 hidrocarburo tales como, pero no limitadas a: etileno o vinilo (-$CH=CH_2$), alilo (-$CH_2CH=CH_2$), ciclopentenilo (-C_5H_7), y 5-hexenilo (-$CH_2CH_2CH_2CH_2CH=CH_2$).

"Alquinilo" es C_2-C_{18} hidrocarburo que contiene átomos de carbono normal, secundario, terciario o cíclico con por lo menos un sitio de insaturación *es decir*, un enlace triple sp carbono-carbono. Ejemplos de radicales alquinilo incluyen fracciones C_2-C_8 hidrocarburo tales como, pero no limitada a: acetilénico (-C≡CH) y propargilo (-CH_2C≡CH).

"Alquileno" se refiere a un radical hidrocarburo de cadena recta o ramificada, o cíclico, saturado de 1-18 átomos de carbono, y que tiene dos centros de radical monovalente derivado por la eliminación de dos átomos de hidrógeno de los mismos o diferentes átomos de carbono de un alcano original. Radicales alquileno típicos incluyen fracciones C_1-C_8 hidrocarburo tales como, pero no limitadas a: metileno (-CH_2-) 1,2-etilo (-CH_2CH_2-), 1,3-propilo (-$CH_2CH_2CH_2$-), 1,4-butilo (-$CH_2CH_2CH_2CH_2$-), y similares.

"Alquenileno" se refiere a un radical hidrocarburo de cadena recta o ramificada, o cíclico, insaturado, de 2-18 átomos de carbono, y que tiene dos centros de radical monovalente derivados por la eliminación de dos átomos de hidrógeno de los mismos o diferentes átomos de carbono de un alqueno original. Radicales alquenileno típicos incluyen fracciones de C_2-C_8 hidrocarburo tales como, pero no se limitan a: 1,2-etileno (-CH=CH-).

"Alquinileno" se refiere a un radical hidrocarburo de cadena recta o ramificada, o cíclico, insaturado de 2-18 átomos de carbono, y que tiene dos centros de radical monovalente derivados por la eliminación de dos átomos de hidrógeno de los mismos o dos diferentes átomos de carbono de un alquino original. Radicales alquinileno típicos incluyen fracciones de C_2-C_8 hidrocarburo tales como, pero no se limitan a: acetileno (-C≡C-), propargilo (-CH_2C≡C-), y 4-pentinilo (-$CH_2CH_2CH_2$C≡C-).

"Arilo", solo o en combinación, significa un radical hidrocarburo aromático monovalente de 6-20 átomos de carbono derivado por la eliminación de un átomo de hidrógeno de un solo átomo de carbono de un sistema de anillo aromático original. Un radical arilo puede contener uno, dos o tres anillos, en donde tales anillos pueden unirse en una manera pendiente, *por ejemplo* bifenilo, o pueden fusionarse, *por ejemplo* naftaleno o antraceno. Algunos grupos arilo se representan en las estructuras ejemplares como "Ar". Grupos arilo típicos incluyen fracciones C_6-C_{12} hidrocarburo tales como, pero no se limitan a, radicales derivados de benceno, benceno sustituido, naftaleno, antraceno, bifenilo, y similares.

"Arilalquilo" de refiere a un radical alquilo acíclico, en el cual uno de los átomos de hidrógeno enlazado a un átomo de carbono, típicamente un terminal o átomo de carbono sp^3, se reemplaza con un radical arilo. Grupos arilalquilo típicos incluyen, pero no se limitan a, bencilo, 2-feniletan-1-ilo, 2-fenileten-1-ilo, naftilmetilo, 2-naftiletan-1-ilo, 2-naftileten-1-ilo, naftobencilo, 2-naftofeniletan-1-ilo y similares. El grupo arilalquilo comprende 6 a 20 átomos de carbono, *por ejemplo* la fracción alquilo, incluidos grupos alcanilo, alquenilo o alquinilo, del grupo arilalquilo tiene 1 a 6 átomos de carbono y la fracción arilo tiene 5 a 14 átomos de carbono.

"Heteroarilalquilo" se refiere a un radical alquilo acíclico, en el cual uno de los átomos de hidrógeno enlazado a un átomo de carbono, típicamente un terminal o átomo de carbono sp^3, se reemplaza con un radical heteroarilo. Grupos heteroarilalquilo típicos incluyen, pero no se limitan a, 2-benzimidazolilmetilo, 2-furiletilo, y similares. El grupo heteroarilalquilo comprende 6 a 20 átomos de carbono, *por ejemplo* la

fracción alquilo, incluidos grupos alcanilo, alquenilo o alquinilo, del grupo heteroarilalquilo tiene 1 a 6 átomos de carbono y la fracción heteroarilo tiene 5 a 14 átomos de carbono y 1 a 3 heteroátomos seleccionados de N, O, P, y S. La fracción heteroarilo del grupo heteroarilalquilo puede ser un monociclo con 3 a 7 miembros de anillo (2 a 6 átomos de carbono, o un biciclo que tiene de 7 a 10 miembros de anillo (4 a 9 átomos de carbono y 1 a 3 heteroátomos seleccionados de N, O, P, y S), por ejemplo: un sistema [4,5], [5,5], [5,6], o [6,6] biciclo.

Grupos alquilo, alquileno, arilo, arilalquilo, y heteroarilalquilo pueden sustituirse donde uno o más átomos de hidrógeno se reemplazan cada uno independientemente con un sustituyente. Sustituyentes típicos incluyen, pero no se limitan a, -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, - $OP(=O)(OR)_2$, $-PC=O)(OR)_2$, $-PO^-_3$, $-PO_3H_2$, -C(=O)R, -C(=O)X, -C(=S)R, $-CO_2R$, $-CO_2$", -C(=S)OR, -C(=O)SR, -C(=S)SR, $-C(=O)NR_2$, $-C(=S)NR_2$, $-C(=NR)NR_2$, donde cada X es independientemente un halógeno: F, Cl, Br, o I; y cada R es independientemente H, C_1-C_{18} alquilo, C_6-C_{20} arilo, C_3-C_{14} heterociclo, grupo protector o fracción profármaco. Análogamente pueden sustituirse grupos alquileno, alquenileno, y alquinileno descritos anteriormente.

"Heteroarilo", también conocido como heterociclo o heterociclilo, se refiere a un radical de sistema de anillo, en el cual uno o más átomos del anillo es un heteroátomo, *por ejemplo* nitrógeno, oxígeno, y azufre. El radical heteroarilo comprende 5 a 14 átomos de carbono y 1 a 3 heteroátomos seleccionados de N, O, P, y S. Un heteroarilo puede ser un monociclo que tiene 3 a 7 miembros de anillo (2 a 6 átomos de carbono y 1 a 3 heteroátomos seleccionados de N, O, P, y S), o un biciclo que tiene 7 a 10 miembros de anillo (4 a 9 átomos de carbono y 1 a 3 heteroátomos seleccionados de N, O, P, y S), por ejemplo: un sistema [4,5], [5,5], [5,6], o [6,6] biciclo. Compuestos heteroarilo se describen en 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, y 28; y J. Am. Chem. Soc. (1960) 82:5566.

"Enlazador" o "enlazar" significa una fracción química que comprende un enlace covalente o una cadena de átomos que une covalentemente un anticuerpo a una fracción de medicamento. En varias incorporaciones, un enlazador se especifica como L. Los enlazadores incluyen un radical divalente tal como un alquileno, un arileno, un heteroarileno, fracciones tales como: $—(CR_2)_nO(CR_2)_n—$, unidades repetitivas de alquiloxi (*por ejemplo* polietilenoxi, PEG, polimetileneoxi) y alquilamino (*por ejemplo* polietilenamino, Jeffamine™); y éster diácido y amidas, incluidos succinato, succinamida, diglicolato, malonato, y caproamida.

El término "quiral" se refiere a moléculas que tienen la propiedad de no superimponibilidad de la contraparte de imagen especular, mientras que el término "aquiral" se refiere a moléculas, que son superimponibles en su contraparte de imagen especular.

El término "estereoisómeros" se refiere a compuestos que tienen constitución química idéntica, pero difieren con respecto al arreglo de los átomos o grupos en el espacio.

"Diastereómero" se refiere a un estereoisómero con dos o más centros de quiralidad y cuyas moléculas no son imágenes especulares uno de otro. Los diastereómeros tienen diferentes propiedades físicas, *por ejemplo* puntos de fusión, puntos de ebullición, propiedades espectrales, y reactividades. Mezclas de diastereómeros pueden separarse bajo procedimientos analíticos de alta resolución, tales como electroforesis y cromatografía.

"Enantiómeros" se refieren a dos estereoisómeros de un compuesto que son imágenes especulares no superimponibles uno de otro.

Las definiciones estereoquímicas y convenciones usadas en esta solicitud siguen generalmente a S. P. Parker, Ed., *McGraw-Hill Dictionary*

of Chemical Terms (1984) McGraw-Hill Book Company, New York; y Eliel, E. and Wilen, *S., Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Muchos compuestos orgánicos existen en formas ópticamente activas, *es decir*, tienen la capacidad de rotar el plano de luz polarizada en el plano. Cuando se describe un compuesto ópticamente activo, los prefijos D y L, o *R* y *S*, se utilizan para denotar la configuración absoluta de la molécula sobre su centro(s) quiral. Los prefijos d y l ó (+) y (-) se emplean para designar el signo de rotación de luz polarizada en el plano por el compuesto, significando (-) ó l que el compuesto es levorrotatorio. Un compuesto con el prefijo (+) o d es dextrorrotatorio. Para una estructura química dada, estos estereoisómeros son idénticos, excepto que son imágenes especulares uno de otro. Un estereoisómero específico puede designarse también como un enantiómero, y una mezcla de tales isómeros se denomina a menudo una mezcla enantiomérica. Una mezcla 50:50 de enantiómeros se denomina una mezcla racémica o un racemato, que puede ocurrir donde no ha habido estereoselección o estereospecificidad en un proceso o reacción química. Los términos "mezcla racémica" y "racemato" se refieren a una mezcla equimolar de dos especies enantioméricas, desprovistas de actividad óptica.

La expresión "sal farmacéuticamente aceptable," tal como se emplea en esta solicitud, se refiere a sales orgánicas o inorgánicas farmacéuticamente aceptables de un ADC. Sales ejemplares incluyen, pero no se limitan a, sulfato, citrato, acetato, oxalato, cloruro, bromuro, yoduro, nitrato, bisulfato, fosfato, fosfato ácido, isonicotinato, lactato, salicilato, citrato ácido, tartrato, oleato, tannato, pantotenato, bitartrato, ascorbato, succinato, maleato, gentisinato, fumarato, gluconato, glucuronato, saccarato, formiato, benzoato, glutamato, metanosulfonato, etanosulfonato, bencenosulfonato, *p*-toluenosulfonato, y sales pamoato (*es decir*, 1,1'-metilen-bis-(2-hidroxi-3-naftoato)). Una sal farmacéuticamente aceptable puede involucrar la inclusión de otra molécula tal como un ión acetato, un ión succinato u otro ión contrario. El ión contrario puede ser cualquier fracción orgánica o inorgánica, que estabiliza la carga en el compuesto

original. Más ún, una sal farmacéuticamente aceptable puede tener en su estructura más de un átomo cargado. Casos donde muchos átomos cargados son parte de una sal farmacéuticamente aceptable pueden tener múltiples iones contrarios. Por lo tanto, una sal farmacéuticamente aceptable puede tener uno o más átomos cargados y/o uno o más iones contrarios.

"Solvato farmacéuticamente aceptable" se refiere a una asociación de uno o más moléculas de disolvente y un ADC. Ejemplos de disolventes que forman solvatos farmacéuticamente aceptables incluyen, pero no se limitan a, agua, isopropanol, etanol, metanol, DMSO, acetato de etilo, ácido acético, y etanolamina.

CONJUGADOS ANTICUERPO-MEDICAMENTO

Los compuestos de la invención incluyen aquellos con utilidad para la actividad antineoplásica. En particular, los compuestos incluyen un anticuerpo conjugado, *es decir*, unidos covalentemente por un enlazador, a una fracción de medicamento, donde el medicamento, cuando no se conjuga con un anticuerpo, tiene un efecto citotóxico o citostático. La actividad biológica de la fracción de medicamento se modula así por conjugación con un anticuerpo. Los conjugados anticuerpo-medicamento (ADC) de la invención puede dispensar selectivamente una dosis efectiva de un agente citotóxico a un tejido tumoral, con lo cual puede lograrse una mayor selectividad, *es decir*, un dosis eficaz más baja.

En una incorporación, la biodisponibilidad del ADC, o un metabolito intracelular del ADC, se mejora en un mamífero, comparada con el compuesto maitansinoide correspondiente solo. Asimismo, la biodisponibilidad del ADC, o un metabolito intracelular del ADC se mejora en un mamífero, cuando se compara con el anticuerpo correspondiente solo (anticuerpo del ADC, sin la fracción de medicamento o enlazador).

En una incorporación, la fracción de medicamento maitansinoide del ADC no se separa del anticuerpo hasta cuando el conjugado anticuerpo-medicamento se une a un receptor de superfice celular o penetra una célula con un receptor de superficie celular, específico para el anticuerpo del

conjugado anticuerpo-medicamento. La fracción de medicamento puede separarse del anticuerpo una vez que el conjugado anticuerpo-medicamento entra a la célula. La fracción de medicamento maitansinoide puede segmentarse intracelularmente, en un mamífero, del anticuerpo del compuesto, o un metabolito intracelular del compuesto, por acción enzimática, hidrólisis, oxidación, u otro mecanismo. Por ejemplo, y en ningún modo se pretende limitar la invención a un mecanismo particular de acción, el átomo de azufre de la fracción de medicamento maitansinoide del ADC puede oxidarse en una sulfona o grupo sulfóxido. Protones en carbonos unidos a la sulfona y el sulfóxido pueden retirarse bajo catálisis general o enzimática dentro de la célula y resultar en una fragmentación de eliminación beta, que segmenta y separa la fracción de medicamento del anticuerpo del ADC. Alternativamente, otros grupos de retiro de electrones, tales como amidas en el enlazador, anticuerpo o medicamento pueden producir mecanismos de segmentación similares dentro de una célula.

Conjugados anticuerpo-medicamento (ADC) pueden representarse por la Fórmula I:

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

o su sal o solvato farmacéuticamente aceptable, en donde:

Ab es un anticuerpo que se une a un receptor ErbB, o que se une a uno o más antígenos asociados a un tumor o receptores de superficie celular seleccionados de (1)-(36):

(1) BMPR1B (proteína morfogenética ósea tipo receptor IB, No. Acceso a Genbank NM_001203);

(2) E16 (LAT1, SLC7A5, No. Acceso a Genbank NM_003486);

(3) STEAP1 (antígeno epitelial de transmembrana de seis hélices de próstata, No. Acceso a Genbank NM_012449);

(4) 0772P (CA125, MUC16, No. Acceso a Genbank AF361486);

(5) MPF (MPF, MSLN, SMR, factor potenciador de megacariocito, mesotelina, No. Acceso a Genbank NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, familia portadora de soluto 34 (fosfato de sodio), miembro 2, transportador 3b de fosfato dependiente de sodio tipo II, No. Acceso a Genbank NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaforina 5b Hlog, dominio sema, siete repeticiones de trombospondina (tipo 1 y similar a tipo 1), dominio de transmembrana (TM) y dominio citoplásmico corto, (semaforina) 5B, No. Acceso a Genbank AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, No. Acceso a Genbank AY358628);

(9) ETBR (receptor tipo B de endotelina, No. Acceso a Genbank AY275463);

(10) MSG783 (RNF124, proteína hipotética FLJ20315, No. Acceso a Genbank NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, gen 1 asociado con cáncer de próstata, proteína 1 asociada con cáncer de próstata, antígeno epitelial de transmembrana de seis hélices de próstata 2, proteína de próstata de transmembrana de seis hélices, No. Acceso a Genbank AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, canal de catión potencial de receptor transitorio, subfamilia M, miembro 4, No. Acceso a Genbank NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, factor de crecimiento derivado de teratocarcinoma, No. Acceso a Genbank NP_003203 ó NM_003212);

(14) CD21 (CR2 (receptor 2 de Complemento) o C3DR (receptor del virus C3d/Epstein Barr) o Hs.73792 No. Acceso a Genbank M26004);

(15) CD79b (CD79B, CD79β, IGb (beta asociada a inmunoglobulina), B29, No. Acceso a Genbank NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAPlA (dominio SH2 que contiene proteína 1a de anclaje fosfatasa), SPAP1B, SPAP1C, No. Acceso a Genbank NM_030764);

(17) HER2 (No. Acceso a Genbank M11730);

(18) NCA (No. Acceso a Genbank M18728);

(19) MDP (No. Acceso a Genbank BC017023);

(20) IL20Rα (No. Acceso a Genbank AF184971);

(21) Brevican (No. Acceso a Genbank AF229053);

(22) EphB2R (No. Acceso a Genbank NM_004442);

(23) ASLG659 (No. Acceso a Genbank AX092328);

(24) PSCA (No. Acceso a Genbank AJ297436);

(25) GEDA (No. Acceso a Genbank AY260763;

(26) BAFF-R (receptor de factor activador de linfocito B, receptor BLyS 3, BR3, NP_443177.1);

(27) CD22 (isoforma CD22-B de receptor de linfocito B, NP-001762.1);

(28) CD79a (CD79A, CD79α, alfa asociada a inmunoglobulina, una proteína específica de linfocito B que interactúa covalentemente con Ig beta (CD79B) y forma un complejo en la superficie con moléculas IgM, transduce una señal involucrada en la diferenciación de linfocitos B, No. Acceso a Genbank NP_001774.1);

(29) CXCR5 (receptor 1 de linfoma 1 de Burkitt, un receptor acoplado a proteína G, que se activa por la quimiocina CXCL13, funciona en migración de linfocitos y defensa humoral, juega un papel en la infección HIV-2 y, quizá, desarrollo de SIDA, linfoma, mieloma, y leucemia, No. Acceso a Genbank NP_001707.1);

(30) HLA-DOB (subunidad Beta de la molécula clase II MHC (antígeno Ia), que une péptidos y los presenta a linfocitos T CD4+, No. Acceso a Genbank NP_002111.1);

(31) P2X5 (canal iónico 5, abierto por acción de ligando P2X, de receptor purinérgico, un canal iónico abierto por ATP extracelular, puede estar involucrado en la transmisión sináptica y neurogénesis; la deficiencia

puede contribuir a la patofisiología de inestabilidad detrusora idiopática, No. Acceso a Genbank NP_002552.2);

(32) CD72 (antígeno CD72 de diferenciación de linfocitos B, Lyb-2, No. Acceso a Genbank NP_001773.1);

(33) LY64 (antígeno 64 de linfocitos (RP105), proteína de membrana tipo I de la familia de repeticiones, rica en leucina (LRR), regula la activación y apoptosis de linfocitos B; la pérdida de función se asocia con actividad patológica incrementada en pacientes con lupus sistémico eritematoso, No. Acceso a Genbank NP_005573.1);

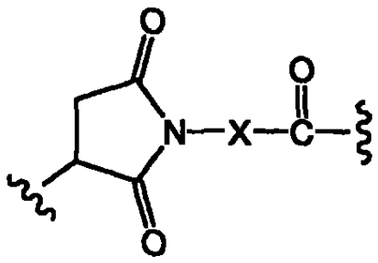
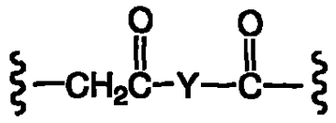
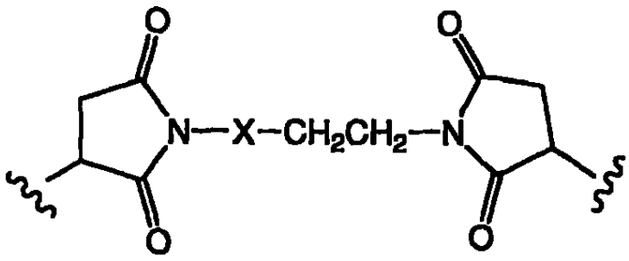
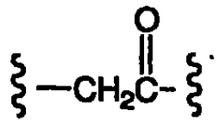
(34) FcRH1 (proteína 1 similar a receptor Fc, un receptor putativo para el dominio Fc de inmunoglobulina que contiene dominios similares a Ig tipo C2 y dominios ITAM, puede tener un papel en la diferenciación de linfocitos B, No. Acceso a Genbank NP_443170.1);

(35) IRTA2 (asociado 2 de translocación de receptor de superfamilia de inmunoglobulina, un inmunorreceptor putativo con posibles papeles en el desarrollo de linfocitos B y génesis de linfoma; la desregulación del gen por translocación ocurre en algunas neoplasias malignas de linfocitos B, No. Acceso a Genbank NP_112571.1); y

(36) TENB2 (proteoglucano de transmembrana putativo, relacionado con la familia EGF/herregulina de factores de crecimiento y folistatina, No. Acceso a Genbank AF179274;

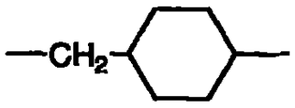
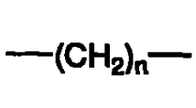
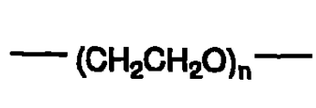
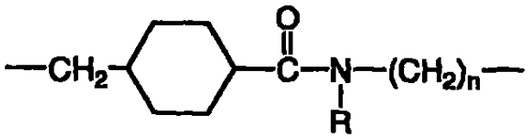
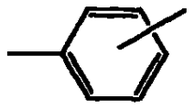
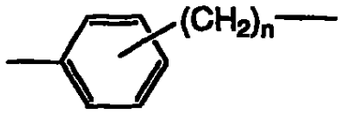
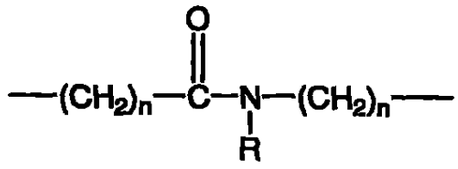
siempre que el anticuerpo no sea TA.1.

L es un enlazador no disulfuro. L incluye, pero no se limita a, las estructuras:

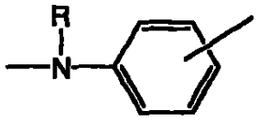
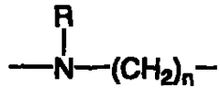





donde las líneas onduladas indican las uniones covalentes a Ab y D;

X es:

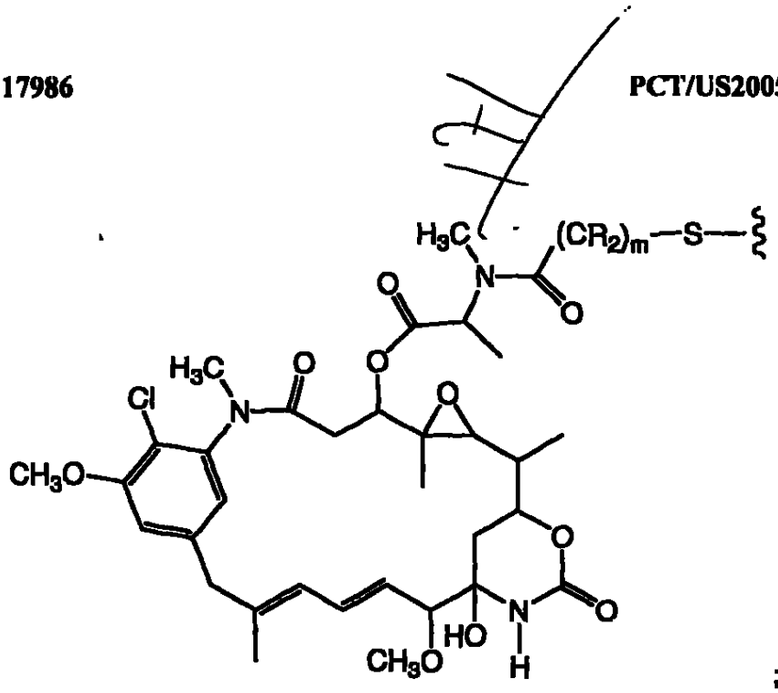








Y es:

R es independientemente H ó C_1-C_6 alquilo; y n es 1 a 12;

D es una fracción de medicamento maitansinoide. Maitansinoides incluyen, pero no se limitan a, la estructura:



en donde la línea ondulada indica la unión covalente a L;

R es independientemente H ó C_1-C_6 alquilo; y

m es 1, 2, ó 3.

La relación medicamento a anticuerpo o carga de medicamento se representa por p para Compuestos de Fórmula I. El valor de carga de medicamento p es 1 a 8. Compuestos de Fórmula I incluyen todas las mezclas de conjugados anticuerpo-medicamento de carga y unión diversa, donde 1, 2, 3, 4, 5, 6, 7, y 8 fracciones de medicamento se unen covalentemente al anticuerpo.

En otra incorporación, Ab es un anticuerpo que se une a uno o más antígenos asociados a tumor o receptores de superficie celular, seleccionados de (1)-(16) y (18)-(36), *es decir*, no a un receptor ErbB, incluido HER2.

ANTICUERPOS

La unidad anticuerpo (Ab-) de Fórmula I incluye dentro de su ámbito cualquier unidad de un anticuerpo que se une, o se asocia reactivamente o se acompleja con un receptor, antígeno u otra fracción receptora, asociada con una población de células diana dada. Un anticuerpo puede ser cualquier proteína o molécula similar a proteína que se une a, se acompleja, o reacciona con, una fracción de una población celular que se busca modificar terapéutica o biológicamente en algún otro sentido. En un aspecto, la unidad anticuerpo actúa para suministrar la fracción de

medicamento maitansinoide a la célula diana particular, con la cual reacciona la unidad anticuerpo. Tales anticuerpos incluyen, pero no se limitan a, proteínas de gran peso molecular tales como, anticuerpos de longitud completa y fragamentos de anticuerpo.

Anticuerpos que comprenden los conjugados anticuerpo-medicamento de la invención conservan preferiblemente la capacidad de unión de antígeno de sus contrapartes tipo natural, nativas. Por consiguiente, anticuerpos de la invención son capaces de unirse, preferiblemente en forma específica, a antígenos. Tales antígenos incluyen, por ejemplo, antígenos asociados con tumores (TAA), proteínas de receptor de superficie celular y otras moléculas de superfice celular, factores reguladores de supervivencia celular, factores reguladores de proliferación celular, moléculas asociadas con (*por ejemplo*, conocidas o sospechosas de contribuir funcionalmente a) desarrollo tisular o diferenciación, linfocinas, citocinas, moléculas involucradas en la regulación del ciclo celular, moléculas involucradas en vasculogénesis y moléculas asociadas con (*por ejemplo*, conocidas o sospechosas de contribuir funcionalmente a) angiogénesis. El antígeno asociado con tumores puede ser un factor de diferenciación de agrupación (*es decir*, una proteína CD). Un antígeno al cual un anticuerpo de la invención es capaz de unirse puede ser un miembro de un subconjunto de una de las categotorías ya mencionadas, en donde el otro subconjunto(s) de dicha categoría comprende otras moléculas/antígenos, que poseen una característica distinta (con respecto al antígeno de interés).

En una incorporación, el anticuerpo de los conjugados anticuerpo-medicamento (ADC) se une específicamente a un receptor codificado por un gen ErbB. El anticuerpo puede unirse específicamente a un receptor ErbB seleccionado de EGFR, HER2, HER3 y HER4. El ADC puede unirse específicamente al dominio extracelular (ECD) del receptor HER2 e inhibir el crecimiento de células tumorales que sobreexpresan el receptor HER2. El anticuerpo del ADC puede ser un anticuerpo monoclonal, *por ejemplo* un anticuerpo monoclonal murino, un anticuerpo quimérico, o un anticuerpo

humanizado. Un anticuerpo humanizado puede ser huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 ó huMAb4D5-8 (trastuzumab). El anticuerpo puede ser un fragmento de anticuerpo, *por ejemplo* un fragmento Fab.

Anticuerpos en los conjugados anticuerpo-medicamento de Fórmula I (ADC) y que pueden ser útiles en el tratamiento de cáncer incluyen, pero no se limitan a, anticuerpos contra receptores de superficie celular y antígenos asociados con tumores (TAA). Tales antígenos asociados con tumores son conocidos en el arte, y pueden prepararse para uso en generar anticuerpos, usando métodos e información que son bien conocidos en el arte. En intentos por descubrir blancos celulares efectivos para diagnosis de cáncer y terapia, los investigadores han buscado identificar polipéptidos transmembrana o asociados en otra forma con tumores, que se expresen específicamente en la superficie de uno o más tipo(s) particular de célula cancerosa, comparada con una o más células no cancerosas, normales. A menudo, tales polipétidos asociados con tumor se expresan más abundantemente en la superficie de células cancerosas, que en la superficie de células no cancerosas. La identificación de tales polipéptidos antígeno de superficie celular, asociados con tumores, ha dado lugar a la capacidad de seleccioanr específicamente células cancerosas diana para destrucción mediante terapia a base de anticuerpo.

Ejemplos de TAA incluyen, pero no se limitan a, Antígenos Asociados con Tumores (1)-(36) enumerados a continuación. Por conveniencia, la información relativa a estos antígenos, todos los cuales son conocidos en el arte, se enumera a continuación e incluye nombres, nombres alternativos, números de acceso a Genbank y referencia(s) primaria, siguiendo la convenciones de identificación de secuencias de proteínas y ácidos nucleicos del Centro Nacional de Información de Biotecnología (NCBI). Secuencias de proteínas y ácidos nucleicos correspondientes a TAA (1)-(36) se encuentran disponibles en bases de datos públicas, tales como GenBank. Antígenos asociados con tumores seleccionados específicamente por anticuerpos incluyen todas las variantes

e isoformas de secuencia de aminoácidos, que poseen por lo menos alrededor de 70%, 80%, 85%, 90%, ó 95% de identidad de secuencia en relación con las secuencias identificadas en las referencias citadas, o que exhiben sustancialmente las mismas propiedades biológicas o características que un TAA con una secuencia hallada en las referencias citadas. Por ejemplo, un TAA con una secuencia de variante está generalmente en capacidad de unirse específicamente a un anticuerpo, que se une específicamente al TAA con la secuencia correspondiente enumerada. Las secuencias y divulgación en la referencia específicamente relacionada aquí se incorporan expresamente por referencia.

ANTÍGENOS ASOCIADOS CON TUMORES (1)-(36):

(1) BMPR1B (proteína morfogenética ósea tipo receptor IB, No. Acceso a Genbank NM_001203) ten DijkeJP., et al., Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Reivindicación 2); WO2003042661 (Reivindicación 12); US2003134790-A1 (Página 38-39); WO2002102235 (Reivindicación 13; Página 296); WO2003055443 (Página 91-92); WO200299122 (Ejemplo 2; Página 528-530); WO2003029421 (Reivindicación 6); WO2003024392 (Reivindicación 2; Fig 112); WO200298358 (Reivindicación 1; Página 183); WO200254940 (Página 100-101); WO200259377 (Página 349-350); WO200230268 (Reivindicación 27; Página 376); WO200148204 (Ejemplo; Fig 4) NP_001194 receptor de proteína morfogenética ósea, tipo IB /pid=NP_001194.1 – Referencias Cruzadas: MIM:603248; NP_001194.1; AY065994

(2) E16 (LAT1, SLC7A5, No. Acceso a Genbank NM_003486)

Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H.W., et al (1992) J. Biol. Chem. 267 (16):11267-11273); WO2004048938 (Ejemplo 2); WO2004032842 (Ejemplo IV); WO2003042661 (Reivindicación 12); WO2003016475 (Reivindicación 1); WO200278524 (Ejemplo 2); WO200299074 (Reivindicación 19; Página 127-129); WO200286443 (Reivindicación 27;

Páginas 222, 393); WO2003003906 (Reivindicación 10; Página 293); WO200264798 (Reivindicación 33; Página 93-95); WO200014228 (Reivindicación 5; Página 133-136); US2003224454 (Fig 3); WO2003025138 (Reivindicación 12; Página 150); US 20050107595; US 20050106644; NP_003477 familia 7 de transportador de soluto 7 (transportador de aminoácido catiónico, sistema y+), miembro 5 /pid=NP_003477.3 - Homo sapiens
Referencias Cruzadas: MIM:600182; NP_003477.3; NM_015923; NM_003486_1

(3) STEAP1 (antígeno epitelial de transmembrana de seis hélices de próstata, No. Acceso a Genbank NM_012449) Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., et al (1999) Proc. Natl. Acad. Sci. U.S.A. 96 (25):14523- 14528); WO2004065577 (Reivindicación 6); WO2004027049 (Fig 1L); EP1394274 (Ejemplo 11); WO2004016225 (Reivindicación 2); WO2003042661 (Reivindicación 12); US2003157089 (Ejemplo 5); US2003185830 (Ejemplo 5); US2003064397 (Fig 2); WO200289747 (Ejemplo 5; Página 618-619); WO2003022995 (Ejemplo 9; Fig 13A, Ejemplo 53; Página 173, Ejemplo 2; Fig 2A);
NP_036581 antígeno epitelial de transmembrana de seis hélices de la próstata, Referencias Cruzadas: MIM:604415; NP_036581.1; NM_012449_1

(4) 0772P (CA125, MUC16, No. Acceso a Genbank AF361486)
J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Reivindicación 14); WO200292836 (Reivindicación 6; Fig 12); WO200283866 (Reivindicación 15; Página 116-121); US2003124140 (Ejemplo 16); US2003091580 (Reivindicación 6); WO200206317 (Reivindicación 6; Página 400-408);
Referencias Cruzadas: GI:34501467; AAK74120.3; AF361486_1

(5) MPF (MPF, MSLN, SMR, factor potenciador de megacariocito, mesotelina, No. Acceso a Genbank NM_005823)
Yamaguchi, N., et al Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. U.S.A. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. U.S.A. 93 (1): 136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995)); WO2003101283 (Reivindicación 14); (WO2002102235 (Reivindicación 13; Página 287-288); WO2002101075 (Reivindicación 4; Página 308-309); WO200271928 (Página 320-321); WO9410312 (Página 52-57);
Referencias Cruzadas: MIM:601051; NP_005814.2; NM_005823_1

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, familia 34 de transportador de soluto (fosfato de sodio), miembro 2, transportador 3b de fosfato dependiente de sodio tipo II, No. Acceso a Genbank NM_006424)
J. Biol. Chem. 277 (22): 19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., et al (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582); WO2004022778 (Reivindicación 2); EP1394274 (Ejemplo 11); WO2002102235 (Reivindicación 13; Página 326); EP875569 (Reivindicación 1; Página 17-19); WO200157188 (Reivindicación 20; Página 329); WO2004032842 (Ejemplo IV); WO200175177 (Reivindicación 24; Página 139-140);
Referencias Cruzadas: MIM:604217; NP_006415.1; NM_006424_1

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaforina 5b Hlog, dominio, siete repeticiones de trombospondina (tipo 1 y similar a tipo 1), dominio transmembrana (TM) y dominio citoplásmico corto, (semaforina) 5B, No. Acceso a Genbank AB040878)
Nagase T., et al (2000) DNA Res. 7 (2):143-150); WO2004000997 (Reivindicación 1); WO2003003984 (Reivindicación 1); WO200206339 (Reivindicación 1; Página 50); WO200188133 (Reivindicación 1; Página 41-43, 48-58); WO2003054152 (Reivindicación 20); WO2003101400

(Reivindicación 11); Acceso: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gen, No. Acceso a Genbank AY358628); Ross et al (2002) Cancer Res. 62:2546-2553; US2003129192 (Reivindicación 2); US2004044180 (Reivindicación 12); US2004044179 (Reivindicación 11); US2003096961 (Reivindicación 11); US2003232056 (Ejemplo 5); WO2003105758 (Reivindicación 12); US2003206918 (Ejemplo 5); EP1347046 (Reivindicación 1); WO2003025148 (Reivindicación 20);
Referencias Cruzadas: GL37182378; AAQ88991.1; AY358628_1

(9) ETBR (receptor tipo B de endotelina, No. Acceso a Genbank AY275463);
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Hum. Genet. 111, 198-206; WO2004045516 (Reivindicación 1); WO2004048938 (Ejemplo 2);
WO2004040000 (Reivindicación 151); WO2003087768 (Reivindicación 1); WO2003016475 (Reivindicación 1); WO2003016475 (Reivindicación 1); WO200261087 (Fig 1); WO2003016494 (Fig 6); WO2003025138 (Reivindicación 12; Página 144); WO200198351 (Reivindicación 1; Página 124-125); EP522868 (Reivindicación 8; Fig 2); WO200177172 (Reivindicación 1; Página 297-299); US2003109676; US6518404 (Fig 3); US5773223 (Reivindicación 1a; Col 31-34); WO2004001004;

(10) MSG783 (RNF124, proteína hipotética FLJ20315, No. Acceso a Genbank NM_017763);
WO2003104275 (Reivindicación 1); WO2004046342 (Ejemplo 2); WO2003042661 (Reivindicación 12); WO2003083074 (Reivindicación 14; Página 61); WO2003018621 (Reivindicación 1); WO2003024392 (Reivindicación 2; Fig 93); WO200166689 (Ejemplo 6);
Referencias Cruzadas: Locus ID:54894; NP_060233.2; NM_017763_1

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, gen 1 asociado con cáncer de próstata, proteína 1 asociada con cáncer de próstata, antígeno epitelial de transmembrana de seis hélices de próstata 2, proteína de próstata de transmembrana de seis hélices, No. Acceso a Genbank AF455138)
Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (Reivindicación 1; Fig 1); WO200272596 (Reivindicación 13; Página 54-55); WO200172962 (Reivindicación 1; Fig 4B); WO2003104270 (Reivindicación 11); WO2003104270 (Reivindicación 16); US2004005598 (Reivindicación 22); WO2003042661 (Reivindicación 12); US2003060612 (Reivindicación 12; Fig 10); WO200226822 (Reivindicación 23; Fig 2); WO200216429 (Reivindicación 12; Fig 10);
Referencias Cruzadas: GL22655488; AAN04080.1; AF455138_1

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, canal de catión potencial de receptor transitorio, subfamilia M, miembro 4, No. Acceso a Genbank NM_017636)
Xu, X.Z., et al., Proc. Natl. Acad. Sci. U.S.A. 98 (19): 10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33): 30813-30820 (2003)); US2003143557 (Reivindicación 4); WO200040614 (Reivindicación 14; Página 100-103); WO200210382 (Reivindicación 1; Fig 9A); WO2003042661 (Reivindicación 12); WO200230268 (Reivindicación 27; Página 391); US2003219806 (Reivindicación 4); WO200162794 (Reivindicación 14; Fig 1A-D);
Referencias Cruzadas: MIM:606936; NP_060106.2; NM_017636_1

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, factor de crecimiento derivado de teratocarcinoma, No. Acceso a Genbank NPJ303203 o NMJ)03212)
Ciccodicola, A., et al., EMBO J. 8 (7): 1987-1991 (1989), Am. J. Hum. Genet. 49 (3):555-565 (1991)); US2003224411 (Reivindicación 1); WO2003083041 (Ejemplo 1); WO2003034984 (Reivindicación 12); WO200288170 (Reivindicación 2; Página 52-53); WO2003024392 (Reivindicación 2; Fig 58); WO200216413 (Reivindicación 1; Página 94-95, 105); WO200222808 (Reivindicación 2; Fig 1); US5854399 (Ejemplo 2; Col 17-18); US5792616 (Fig 2);
Referencias Cruzadas: MIM:187395; NP_003203.1; NM_003212_1

(14) CD21 (CR2 (receptor 2 de Complemento) o C3DR (receptor de virus C3d/Epstein Barr) o Hs.73792 No. Acceso a Genbank M26004)
Fujisaku et al (1989) J. Biol. Chem. 264 (4):2118-2125); Weis J.J., et al., J. Exp. Med. 167, 1047-1066, 1988; Moore M., et al., Proc. Natl. Acad. Sci. U.S.A. 84, 9194-9198, 1987; Barel M., et al., Mol. Immunol. 35, 1025-1031, 1998; Weis J.J., et al., Proc. Natl. Acad. Sci. U.S.A. 83, 5639-5643, 1986; Sinha S.K., et al (1993) J. Immunol. 150, 5311-5320; WO2004045520 (Ejemplo 4); US2004005538 (Ejemplo 1);

WO2003062401 (Reivindicación 9); WO2004045520 (Ejemplo 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Reivindicación 1);
Acceso: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) CD79b (CD79B, CD79β, IGb (beta asociada con inmunoglobulina), B29, No. Acceso a Genbank NM_000626 ó 11038674) Proc. Natl. Acad. Sci. U.S.A. (2003) 100 (7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller et al (1992) Eur. J. Immunol. 22 (6): 1621-1625); WO2004016225 (Reivindicación 2, Fig 140); WO2003087768, US2004101874 (Reivindicación 1, Página 102); WO2003062401 (Reivindicación 9); WO200278524 (Ejemplo 2); US2002150573 (Reivindicación 5, Página 15); US5644033; WO2003048202 (Reivindicación 1, Páginas 306 y 309); WO 99/558658, US6534482 (Reivindicación 13, Fig 17A/B); WO200055351 (Reivindicación 11, Páginas 1145-1146);
Referencias Cruzadas: MIM:147245; NP_000617.1; NM_000626_1

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (dominio SH2 que contiene proteína 1a de anclaje fosfatasa), SPAP1B, SPAP1C, No. Acceso a Genbank NMJB0764, AY358130)
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Referencias Cruzadas: MIM:606509; NP_110391.2; NM_030764_1

(17) HER2 (ErbB2, No. Acceso a Genbank M11730)
Coussens L., et al., Science (1985) 230(4730): 1132-1139); Yamamoto T., et al., Nature 319, 230-234, 1986; Semba K., et al., Proc. Natl. Acad. Sci. U.S.A. 82, 6497-6501, 1985; Swiercz J.M., et al., J. Cell Biol. 165, 869-

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Acceso: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

(18) NCA (CEACAM6, No. Acceso a Genbank M18728);

Barnett T., et al., Genomics 3, 59-66, 1988; Tawaragi Y., et al., Biochem. Biophys. Res. Commun. 150, 89-96, 1988; Strausberg R.L., et al., Proc. Natl. Acad. Sci. U.S.A. 99: 16899-16903, 2002; WO2004063709; EP1439393 (Reivindicación 7); WO2004044178 (Ejemplo 4); WO2004031238; WO2003042661 (Reivindicación 12); WO200278524 (Ejemplo 2); WO200286443 (Reivindicación 27; Página 427); WO200260317 (Reivindicación 2);

Acceso: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;

(19) MDP (DPEP1, No. Acceso a Genbank BC017023)

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Referencias Cruzadas: MIM: 179780; AAH17023.1; BC017023_1

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Acceso: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) Brevican (BCAN, BEHAB, No. Acceso a Genbank AF229053)
Gary S.C., et al., Gene 256, 139-147, 2000; Clark H.F., et al., Genome Res. 13, 2265-2270, 2003; Strausberg R.L., et al., Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; US2003186372 (Reivindicación 11); US2003186373 (Reivindicación 11); US2003119131 (Reivindicación 1; Fig 52); US2003119122 (Reivindicación 1; Fig 52); US2003119126 (Reivindicación 1); US2003119121 (Reivindicación 1; Fig 52); US2003119129 (Reivindicación 1); US2003119130 (Reivindicación 1); US2003119128 (Reivindicación 1; Fig 52); US2003119125 (Reivindicación 1); WO2003016475 (Reivindicación 1); WO200202634 (Reivindicación 1);

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, No. Acceso a Genbank NM_004442)
Chan, J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000)); WO2003042661 (Reivindicación 12); WO200053216 (Reivindicación 1; Página 41); WO2004065576

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(23) ASLG659 (B7h, No. Acceso a Genbank AX092328)
US20040101899 (Reivindicación 2); WO2003104399 (Reivindicación 11); WO2004000221 (Fig 3); US2003165504 (Reivindicación 1); US2003124140 (Ejemplo 2); US2003065143 (Fig 60); WO2002102235 (Reivindicación 13; Página 299); US2003091580 (Ejemplo 2); WO200210187 (Reivindicación 6; Fig 10); WO200194641 (Reivindicación 12; Fig 7b); WO200202624 (Reivindicación 13; Fig 1A-1B); US2002034749 (Reivindicación 54; Página 45-46); WO200206317 (Ejemplo 2; Página 320-321, Reivindicación 34; Página 321-322); WO200271928 (Página 468-469); WO200202587 (Ejemplo 1; Fig 1); WO200140269 (Ejemplo 3; Páginas 190-192); WO200036107 (Ejemplo 2; Página 205-207); WO2004053079 (Reivindicación 12); WO2003004989 (Reivindicación 1); WO200271928 (Página 233-234, 452-453); WO 0116318;

(24) PSCA (precursor de antígeno de célula madre de próstata, No. Acceso a Genbank AJ297436)
Reiter R.E., et al., Proc. Natl. Acad. Sci. U.S.A. 95, 1735-1740, 1998; Gu Z., et al., Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-788; WO2004022709; EP1394274 (Ejemplo 11); US2004018553 (Reivindicación 17); WO2003008537 (Reivindicación 1); WO200281646 (Reivindicación 1; Página 164); WO2003003906 (Reivindicación 10; Página 288); WO200140309 (Ejemplo 1; Fig 17); US2001055751 (Ejemplo 1; Fig 1b); WO200032752 (Reivindicación 18; Fig 1); WO9851805 (Reivindicación 17; Página 97); WO9851824 (Reivindicación 10; Página 94); WO9840403 (Reivindicación 2; Fig 1B); Acceso: 043653; EMBL; AF043498; AAC39607.1.

(25) GEDA (No. Acceso a Genbank AY260763);
AAP14954 lipoma HMGIC proteína tipo pareja de fusión /pid=AAP14954.1 – Especie Homo sapiens: Homo sapiens (humana) WO2003054152 (Reivindicación 20); WO2003000842 (Reivindicación 1); WO2003023013 (Ejemplo 3, Reivindicación 20); US2003194704 (Reivindicación 45);
Referencias Cruzadas: GI:30102449; AAP14954.1; AY260763_1

(26) BAFF-R (receptor de factor activador de linfocitos B, receptor 3 BLyS 3, BR3, No. Acceso a Genbank AF116456); BAFF receptor /pid=NP_443177.1 - Homo sapiens
Thompson, J.S., et al., Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611; WO2003045422 (Ejemplo; Página 32-33); WO2003014294 (Reivindicación 35; Fig 6B); WO2003035846 (Reivindicación 70; Página 615-616); WO200294852 (Col 136-137); WO200238766 (Reivindicación 3; Página 133); WO200224909 (Ejemplo 3; Fig 3);
Referencias Cruzadas: MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) CD22 (isoforma CD22-B de receptor de linfocitos B, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814, No. Acceso a Genbank AK026467);
Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Reivindicación 1; Fig 1);
Referencias Cruzadas: MIM:107266; NP_001762.1; NM_001771_1

(28) CD79a (CD79A, CD79α, alfa asociada a inmunoglobulina, una proteína específica de linfocitos B, que interactúa covalentemente con Ig beta (CD79B) y forma un complejo en la superficie con moléculas IgM, transduce una señal involucrada en la diferenciación de linfocitos B) PROTEIN SEQUENCE Full mpggpgv...dvqlekp (1..226; 226 aa), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2, No. Acceso a

Genbank NP_001774.10) WO2003088808, US20030228319; WO2003062401 (Reivindicación 9); US2002150573 (Reivindicación 4, Páginas 13-14); WO9958658 (Reivindicación 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha et al (1992) J. Immunol. 148(5): 1526-1531; Mueller et al (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et al (1994) Immunogenetics 40(4):287- 295; Preud'homme et al (1992) Clin. Exp. Immunol. 90(1): 141-146; Yu et al (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al (1988) EMBO J. 7(11):3457-3464;

(29) CXCR5 (receptor 1 de linfoma de Burkitt, un receptor acoplado a proteína G que se activa por la quimiocina CXCL13, funciona en la migración de linfocitos y defensa humoral, juega un papel en la infección HIV-2 y, quizá, el desarollo de SIDA, Linfoma, mieloma, y leucemia) PROTEIN SEQUENCE Full mnypltl...atslttf (1..372; 372 aa), pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, No. Acceso a Genbank NP_001707.1)
WO2004040000; WO2004015426; US2003105292 (Ejemplo 2); US6555339 (Ejemplo 2); WO200261087 (Fig 1); WO200157188 (Reivindicación 20, Página 269); WO200172830 (Páginas 12-13); WO200022129 (Ejemplo 1, Páginas 152-153, Ejemplo 2, Páginas 254-256); WO9928468 (Reivindicación 1, Página 38); US5440021 (Ejemplo 2, Col 49-52); WO9428931 (Páginas 56-58); WO9217497 (Reivindicación 7, Fig 5); Dobner et al (1992) Eur. J. Immunol. 22:2795- 2799; Barella et al (1995) Biochem. J. 309:773-779;

(30) HLA-DOB (subunidad beta de molécula clase II MHC (antígeno Ia) que une péptidos y los presenta a linfocitos T CD4+) PROTEIN SEQUENCE Full mgsgwvp...vllpqsc (1..273; 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, No. Acceso a Genbank NP_002111.1)
Tonnelle et al (1985) EMBO J. 4(11):2839-2847; Jonsson et al (1989) Immunogenetics 29(6):411-413; Beck et al (1992) J. Mol. Biol. 228:433-

441; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al (1987) J. Biol. Chem. 262:8759-8766; Beck et al (1996) J. Mol. Biol. 255:1-13; Naruse et al (2002) Tissue Antigens 59:512-519; WO9958658 (Reivindicación 13, Fig 15); US6153408 (Col 35-38); US5976551 (Col 168-170); US6011146 (Col 145-146); Kasahara et al (1989) Immunogenetics 30(1):66-68; Larhammar et al (1985) J. Biol. Chem. 260(26):14111-14119;

(31) P2X5 (canal iónico 5, abierto por acción de ligando P2X, de receptor purinérgico, un canal iónico abierto por ATP extracelular, puede estar involucrado en la transmisión sináptica y neurogénesis; la deficiencia puede contribuir a la patofisiología de inestabilidad detrusora idiopática) PROTEIN SEQUENCE Full mgqagck...lephrst (1..422; 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, No. Acceso a Genbank NP_002552.2)
Le et al (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (Reivindicación 10); Touchman et al (2000) Genome Res. 10:165-173; WO200222660 (Reivindicación 20); WO2003093444 (Reivindicación 1); WO2003087768 (Reivindicación 1); WO2003029277 (Página 82);

(32) CD72 (antígeno CD72 de diferenciación de linfocitos B, Lyb-2) PROTEIN SEQUENCE Full maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, No. Acceso a Genbank NP_001773.1) WO2004042346 (Reivindicación 65); WO2003026493 (Páginas 51-52, 57-58); WO200075655 (Páginas 105-106); Von Hoegen et al (1990) J. Immunol. 144(12):4870-4877; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

(33) LY64 (antígeno 64 de linfocitos (RP105), proteína de membrana tipo I de la familia de repeticiones, rica en leucina (LRR), regula la activación y apoptosis de linfocitos B; la pérdida de función se asocia con actividad

patológica incrementada en pacientes con lupus sistémico eritematoso) PROTEIN SEQUENCE Full mafdvsc...rwkyqhi (1..661; 661 aa), pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, No. Acceso a Genbank NP_005573.1)
US2002193567; WO9707198 (Reivindicación 11, Páginas 39-42); Miura et al (1996) Genomics 38(3):299-304; Miura et al (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (Reivindicación 8, Páginas 57-61); WO200012130 (Páginas 24- 26);

(34) FcRH1 (proteína 1 tipo receptor Fc, un receptor putativo para el dominio Fc de inmunoglobulina que contiene dominios similares a Ig tipo C2 y dominios ITAM, puede tener un papel en la diferenciación de linfocitos B) PROTEIN SEQUENCE Full mlprlll...vdyedam (1..429; 429 aa), pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, No. Acceso a Genbank NP_443170.1)
WO2003077836; WO200138490 (Reivindicación 6, Fig 18E-1-18-E-2); Davis et al (2001) Proc. Natl. Acad. Sci USA 98(17):9772-9777; WO2003089624 (Reivindicación 8); EP1347046 (Reivindicación 1); WO2003089624 (Reivindicación 7);

(35) IRTA2 (asociado 2 de translocación de receptor de superfamilia de inmunoglobulina, un inmunorreceptor putativo con posibles papeles en el desarrollo de linfocitos B y génesis de linfoma; la desregulación del gen por translocación ocurre en algunas neoplasias malignas de linfocitos B) PROTEIN SEQUENCE Full mllwvil...assaphr (1..977; 977 aa), pI: 6.88 MW: 106468 TM: 1 [P] Gene Chromosome: 1q21, No. Acceso a Genbank Human:AF343662, AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse:AK089756, AY158090, AY506558; NP_112571.1
WO2003024392 (Reivindicación 2, Fig 97); Nakayama et al (2000) Biochem. Biophys. Res. Commun. 277(1): 124-127; WO2003077836; WO200138490 (Reivindicación 3, Fig 18B-1-18B-2);

(36) TENB2 (TMEFF2, tomorregulina, TPEF, HPP1, TR, proteoglucano de transmembrana putativo, relacionado con la familia EGF/herregulina de factores de crecimiento y folistatina) PROTEIN SEQUENCE Full mvlwesp...rastrli (1..374; 374 aa, NCBI Acceso: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; No. Acceso a Genbank AF179274; AY358907, CAF85723, CQ782436
WO2004074320 (SEQ ID NO 810); JP2004113151 (SEQ ID NOS 2, 4, 8); WO2003042661 (SEQ ID NO 580); WO2003009814 (SEQ ID NO 411); EP1295944 (Páginas 69-70); WO200230268 (Página 329); WO200190304 (SEQ ID NO 2706); US2004249130; US2004022727; WO2004063355; US2004197325; US2003232350; US2004005563; US2003124579; US 6410506; US 66420061; Horie et al (2000) Genomics 67:146-152; Uchida et al (1999) Biochem. Biophys. Res. Commun. 266:593-602; Liang et al (2000) Cancer Res. 60:4907-12; Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2): 178-84.

PRODUCCIÓN DE ANTICUERPOS

Se han empleado varios métodos para producir anticuerpos monoclonales (MAbs). La tecnología de hibridoma, que se refiere a una línea celular clonada que produce un tipo único de anticuerpo, usa las células de varias especies, incluidos ratones (murino), hámsters, ratas, y humanos. Otros métodos para preparar MAbs, incluidos anticuerpos quiméricos y humanizados, utiliza ingeniería genética, *es decir*, técnicas de ADN recombinante.

Anticuerpos policlonales pueden cultivarse en animales por múltiples inyecciones subcutáneas (sc) o intraperitoneales (ip) del antígeno relevante y un coadyuvante. Los anticuerpos monoclonales se obtienen de una población de anticuerpos sustancialmente homogéneos, *es decir*, los anticuerpos individuales que comprenden la población son idénticos,

excepto por posibles mutaciones de ocurrencia natural, que pueden estar presentes en cantidades menores.

Se han descrito igualmente estirpes celulares de mieloma humano y heteromieloma ratón-humano para la producción de anticuerpos monoclonales humanos (Kozbor, (1984) J. Immunol., 133:3001, y Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). El medio de cultivo en el cual crecen las células de hibridoma se analiza con respecto a la producción de anticuerpos monoclonales dirigidos contra el antígeno. La especificidad de unión de los anticuerpos monoclonales, producidos por células de hibridoma, se determina por inmunoprecipitación o por análisis de unión *in vitro*, tal como radioinmunoanálisis (RIA) o enzimoinmunoanálisis absorbente (ELISA). La afinidad de unión del anticuerpo monoclonal puede determinarse, por ejemplo, por el análisis Scatchard de Munson et al (1980) Anal. Biochem., 107:220.

ADN que codifica los anticuerpos monoclonales se aísla fácilmente y se secuencia usando procedimientos convencionales (*por ejemplo*, usando sondas de oligonucleótidos que son capaces de fijarse específicamente a genes, que codifican las cadenas ligera y pesada de anticuerpos murinos). Las células de hibridoma sirven como fuente de tal ADN. Una vez aislado, el ADN puede ponerse en vectores de expresión, los cuales se transfectan luego en células hospederas tales como células de *E. coli*, células COS de simios, células de ovario de hámster de la China (CHO), o células de mieloma que, de otra forma, no producen proteína de anticuerpo, para obtener la síntesis de anticuerpos monoclonales en las células hospederas recombinantes (US 2005/0048572; US 2004/0229310). Artículos de reseña sobre la expresión recombinante en bacterias de ADN que codifica el anticuerpo incluyen Skerra et al (1993) Curr. Opinion in Immunol., 5:256-262 y Plückthun (1992) Immunol. Revs., 130: 151-188.

En una incorporación adicional, anticuerpos o fragmentos de anticuerpo pueden aislarse de bibliotecas de fagos de anticuerpos, generados usando las técnicas descritas en McCafferty et al 1990) Nature,

348: 552-554. Clackson et al (1991) Nature, 352: 624-628; y Marks et al (1991) J. Mol. Biol., 222:581-597 describen el aislamiento de anticuerpos humanos y murinos, respectivamente, utilizando bibliotecas de fagos. Publicaciones subsiguientes describen la producción de anticuerpos humanos de alta afinidad (rango nM) por entremezcla de cadenas (***"chain shuffling"***) (Marks et al (1992) Bio/Technology, 10:779-783), así como infección combinatoria y recombinación *in vivo* como estrategia para construir bibliotecas muy grandes de fagos (Waterhouse et al (1993) Nuc. Acids. Res., 21: 2265-2266). Por lo tanto, éstas técnicas son alternativas viables a las técnicas tradicionales de hibridoma de anticuerpos monoclonales para el aislamiento de anticuerpos monoclonales.

Del mismo modo puede modificarse el ADN, por ejemplo, sustituyendo la secuencia codificante para los dominios constantes humanos de cadena ligera y pesada en lugar de las secuencias murinas homólogas (US 4816567; y Morrison et al (1984) Proc. Natl Acad. Sci. USA, 81: 6851), o juntando covalentemente a la secuencia codificante de inmunoglobulina la totalidad o parte de la secuencia codificante para un polipéptido no inmunoglobulina.

Típicamente tales polipéptidos no inmunoglobulina se sustituyen por los dominios constantes de un anticuerpo, o se sustituyen por los dominios variables de un sitio combinante de antígeno de un anticuerpo para crear un anticuerpo bivalente quimérico, que comprende un sitio combinante de antígeno con especificidad para un antígeno, y otro sitio combinante de antígeno con especificidad para un antígeno diferente.

Sigue una descripción en cuanto a técnicas ejemplares para la producción de los anticuerpos (Ab), usados en los conjugados anticuerpo-medicamento (ADC) de la presente invención. La producción de anticuerpos se ilustrará con referencia a anticuerpos anti-ErbB2, pero será evidente para aquellas personas expertas en el arte que anticuerpos a otros miembros de la familia de receptor ErbB, así como cualquier otro receptor o antígeno asociado con tumor o diana, pueden producirse y modificarse en una manera similar.

El antígeno ErbB2 a usarse para la producción de anticuerpos puede ser, *por ejemplo*, una forma soluble del dominio extracelular de ErbB2 o una porción del mismo, que contiene el epítopo deseado. Alternativamente, células que expresan ErbB2 en su superficie celular, *por ejemplo* células NIH-3T3 transformadas para sobreexpresar ErbB2; o una línea celular de carcinoma tal como células SK-BR-3 (Stancovski et al (1991) PNAS (USA) 88:8691-8695), pueden utilizarse para generar anticuerpos. Otras formas de ErbB2 útiles para generar anticuerpos serán evidentes a los expertos en el arte.

Ejemplo 1 describe la producción de un anticuerpo anti-ErbB2 humanizado ejemplar. El anticuerpo humanizado puede comprender, por ejemplo, residuos de región hipervariable no humana, incorporados en un dominio de cadena pesada variable humana y pueden comprender además una sustitución de región marco (FR) en una posición seleccionada del grupo conformado por 69H, 71H y 73H, utilizando el sistema de numeración de dominio variable divulgado en Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). En una incorporación, el anticuerpo humanizado comprende sustituciones FR en dos posiciones, o en todas, 69H, 71H y 73H.

Como una alternativa a la humanización, pueden generarse anticuerpos humanos. Por ejemplo, actualmente es posible producir animales transgénicos (*por ejemplo*, ratones) que son capaces, luego de la inmunización, de producir un completo repertorio de anticuerpos humanos en la ausencia de producción de inmunoglobulina endógena (Jakobovits et al (1993) Proc. Natl. Acad. Sci. USA, 90:2551; Jakobovits et al (1993) Nature 362:255-258; Bruggermann et al (1993) Year in Immuno. 7:33; y US 5591669; US 5589369; US 5545807).

Alternativamente, tecnología de exposición en fago (McCafferty et al (1990) Nature 348:552-553) puede usarse para producir anticuerpos humanos y fragmentos de anticuerpo *in vitro*, a partir de repertorios de genes de dominio variable (V) de inmunoglublina de donantes no

inmunizados (Johnson, Kevin S. and Chiswell, David J. (1993) *Current Opinion in Structural Biology* 3:564-571). Puede construirse un repertorio de genes V de donantes no inmunizados humanos y, esencialmente, pueden aislarse anticuerpos a un arreglo diverso de antígenos (incluidos auto-antígenos) (Marks et al (1991) J. Mol. Biol. 222:581-597, o Griffith et al (1993) EMBO J. 12:725-734; US 5565332; US 5573905). Anticuerpos humanos pueden generarse igualmente por linfocitos B activados *in vitro* (US 5567610; US 5229275). Se describen anticuerpos anti-ErbB2 humanos (US 5772997 y WO 97/00271.

Se han desarrollado varias técnicas para la producción de fragmentos de anticuerpo. Tradicionalmente, estos fragmentos se derivaron por digestión proteolítica de anticuerpos intactos (véase Morimoto et al (1992) Journal of Biochemical and Biophysical Methods 24:107-117; y Brennan et al (1985) Science 229:81). Fragmentos de anticuerpo pueden producirse también directamente por células hospederas recombinantes y las bibliotecas de fagos de anticuerpos, discutidas anteriormente. Fragmentos Fab'-SH pueden recuperarse directamente de *E. coli* y acoplarse químicamente para formar fragmentos $F(ab')_2$ (Carter et al (1992) Bio/Technology 10:163-167). De acuerdo con otra metodología, los fragmentos $F(ab')_2$ pueden aislarse directamente de cultivo de células hospederas recombinantes. Otras técnicas para la producción de fragmentos de anticuerpos serán evidentes al profesional experto. En otras incorporaciones, el anticuerpo de elección es un fragmento Fv de cadena única (scFv). Véase WO 93/16185; US 5571894; y US 5587458. El fragmento de anticuerpo puede ser igualmente un "anticuerpo lineal", *por ejemplo*, como se describe en US 5641870. Tales fragmentos de anticuerpo lineal pueden ser monoespecíficos o bioespecíficos.

Anticuerpos bioespecíficos con especificidades de unión para, por lo menos, dos epítopos diferentes (Millstein et al (1983), Nature 305:537-539) pueden fijarse a dos diferentes epítopos de la proteína ErbB. Otros anticuerpos similares pueden combinar un sitio de unión ErbB, con sitio(s) de unión para EGFR, ErbB3 y/o ErbB4. Alternativamente, un brazo anti-

ErbB puede combinarse con un brazo que se fije a una molécula desencadenante en un leucocito tal como una molécula de receptor de leucocito T (*por ejemplo*, CD2 ó CD3), o receptores Fc para IgG (FcγR), tales como FcγRI (CD64), FcγRII (CD32) y FcγRIII (CD 16), de modo que se enfoquen mecanismos de defensa a la célula que expresa ErbB. Anticuerpos bioespecíficos pueden usarse además para localizar agentes citotóxicos a células que expresan ErbB2 (WO 96/16673; US 5837234; WO98/02463; US 5821337). Métodos de purificación para anticuerpos biespecíficos han sido divulgados (WO 93/08829; Traunecker et al (1991) EMBO J. 10:3655-3659; WO 94/04690; Suresh et al (1986) *Methods in Enzymology* 121:210; US 5731168). Anticuerpos bioespecíficos pueden producirse usando cierres de leucina (***"leucine zippers"***). Kostelny et al (1992) J. Immunol., 148(5):1547-1553), y dímeros Fv de cadena única (sFv) (Gruber et al (1994) J. Immunol. 152:5368).

Se han descrito igualmente técnicas para generar anticuerpos biespecíficos de fragmentos de anticuerpo, tales como usando enlazamiento químico, en donde anticuerpos intactos se segmentan proteolíticamente para generar fragmentos $F(ab')_2$ (Brennan et al (1985) Science 229:81). Los fragmentos Fab'-SH pueden recuperarse de *E. coli* y acoplarse químicamente para formar anticuerpos biespecíficos (Shalaby et al (1992) J. Exp. Med. 175:217-225. La tecnología de "diacuerpo" provee un método alternativo para elaborar fragmentos de anticuerpos bioespecíficos (Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448).

Se contemplan anticuerpos con más de dos valencias. Anticuerpos "Octopus", multivalentes con tres o más sitios de unión de antígeno y dos o más dominios variables, pueden producirse fácilmente por expresión recombinante de ácido nucleico que codifica las cadenas polipeptídicas del anticuerpo (US 2002/0004586; WO 01/77342). Por ejemplo, pueden prepararse anticuerpos triespecíficos (Tutt et al (1991) J. Immunol. 147:60.

Se contempla(n) modificación (modificaciones) de secuencias de aminoácidos de anticuerpos. Por ejemplo, se contemplan mutantes y varias isoformas de anticuerpos, que se unen a antígenos asociados con tumores,

para mejorar la afinidad de unión y/u otras propiedades biológicas del anticuerpo. Variantes de secuencia de aminoácidos de un anticuerpo se preparan introduciendo cambios de nucleótido apropiados en el ácido nucleico que codifica el anticuerpo, o mediante síntesis peptídica. Tales modificaciones incluyen, por ejemplo, eliminaciones, y/o inserciones en y/o sustituciones, de residuos dentro de las secuencias de aminoácidos del anticuerpo. Se realiza cualquier combinación de eliminación, inserción y sustitución para llegar al constructo final, siempre que el constructo final posea las características deseadas. Los cambios de aminoácidos pueden alterar asimismo procesos pos-traduccionales del anticuerpo, tales como cambiar el número o posición de sitios de glucosilación.

Un método útil para la identificación de ciertos residuos o regiones del anticuerpo, que son sitios preferidos para la mutagénesis se denomina "mutagénesis dirigida de rastreo de alanina" [***"alanine-scanning mutagenesis"***] (Cunningham y Wells (1989) Science, 244:1081-1085), donde se identifica un residuo de aminoácidos o grupo de residuos diana (*por ejemplo*, residuos cargados tales como arg, asp, his, lys, y glu) y se reemplazan por un aminoácido neutro o cargado negativamente, tal como alanina o polialanina, para optimizar la interacción de los aminoácidos con antígeno. Inserciones de secuencia de aminoácidos incluyen fusiones de terminal amino y/o carboxilo, que varían en longitud desde un residuo hasta polipéptidos, que contienen cien o más residuos, así como inserciones intrasecuencia de residuos de aminoácidos únicos o múltiples. Ejemplos de inserciones de terminal incluyen un anticuerpo anti-ErbB con un residuo metionilo N-terminal o el anticuerpo fusionado a un polipéptido citotóxico. Otras variantes de inserción de la molécula del anticuerpo anti-ErbB incluyen la fusión al término N o C del anticuerpo anti-ErbB de una enzima (*por ejemplo* para ADEPT: Tietze et al (2003) Current Pharm. Design 9:2155-2175), o un polipéptido que incrementa la semivida del anticuerpo, tal como un péptido de unión de albúmina.

La unión plasma-proteína puede ser un medio efectivo de mejorar las propiedades farmacocinéticas de moléculas de corta vida. La albúmina es

la proteína más abundante en el plasma. Péptidos de unión de albúmina sérica (ABP) pueden alterar la farmacodinámica de proteínas de dominio activo fusionado, incluida la alteración de captación de tejido, penetración y difusión. Estos parámetros farmacodinámicos pueden modularse por selección específica de la secuencia peptídica apropiada de unión de albúmina sérica (US 20040001827). Una serie de péptidos de unión de albúmina se identificó por cribado de exposición de fago (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). Los compuestos de la invención incluyen secuencias ABP enseñadas por: (i) Dennis et al (2002) J Biol Chem. 277:35035-35043 en las Tablas III y IV, Página 35038; (ii) US 20040001827 en [0076] SEQ ID NOS: 9-22; y (iii) WO 01/45746 en Páginas 12- 13, SEQ ID NOS: z1-z14, cuya totalidad se incorporan en esta solicitud por referencia.

La secuencia de aminoácidos se altera usualmente modificando la secuencia de ácidos nucleicos subyacente. Moléculas de ácido nucleico, que codifican variantes de secuencia de aminoácidos del anticuerpo, se preparan por una variedad de métodos conocidos en el arte. Estos métodos incluyen, pero no se limitan a, aislamiento de una fuente natural (en el caso de variantes de secuencia de aminoácidos de ocurrencia natural), o preparación por mutagénesis mediada por oligonucleótido (o dirigida al sitio), mutagénesis de PCR, y mutagénesis de casete de una variante previamente preparada o una versión no variante del anticuerpo. Los sitios de máximo interés para mutagénesis sustitutiva incluyen las regiones hipervariables, pero se contemplan también alteraciones FR.

Modificaciones sustanciales en las propiedades biológicas del anticuerpo se llevan a cabo seleccionando sustituciones que difieren significativamente en su efecto sobre el mantenimiento de (a) la estructura del esqueleto polipeptídico en el área de la substitución, por ejemplo, como una hoja o conformación helicoidal, (b) la carga de hidrofobicidad de la molécula en el sitio diana, o (c) la parte principal de la cadena lateral.

Residuos de ocurrencia natural se dividen en grupos basados en propiedades comunes de cadena lateral:

(1) hidrófobo: norleucina, met, ala, val, leu, ile;

(2) hidrófilo neutro: cys, ser, thr;

(3) ácido: asp, glu;

(4) básico: asn, gln, his, lys, arg;

(5) residuos que influyen en la orientación de la cadena: gly, pro; y

(6) aromático: trp, tyr, phe.

Sustituciones no conservadoras incluirán intercambiar un miembro de una de estas clases por otra clase.

Puede sustituirse además cualquier residuo de cisteína no involucrado en mantener la conformación apropiada del anticuerpo, generalmente con serina, para mejorar la estabilidad oxidativa de la molécula y prevenir entrelazamiento cruzado aberrante. Inversamente, enlace(s) de cisteína puede(n) agregarse al anticuerpo para mejorar sus estabilidad (particularmente donde el anticuerpo es un fragmento de anticuerpo, tal como un fragmento Fv).

Para incrementar la semivida sérica del anticuerpo, se puede incorporar un epítopo de unión a receptor de salvamento al antagonista (especialmente un fragmento de anticuerpo), como se describe en US 5739277, por ejemplo. Tal como se emplea en esta solicitud, el término "epítopo de unión a receptor de salvamento" se refiere a un epítopo de la región Fc de una molécula IgG (*por ejemplo*, IgG_1, IgG_2, IgG_3, o IgG_4) que es responsable de aumentar la semivida sérica *in vivo* de la molécula IgG (US 2003/0190311, US6821505; US 6165745; US 5624821; US 5648260; US 6165745; US 5834597).

Variantes de glucosilación de anticuerpos son variantes en las cuales se altera el patrón de glucosilación de un anticuerpo. Por alterar se da a entender eliminar una o más fracciones carbohidrato encontradas en el anticuerpo, agregar una o más fracciones carbohidrato al anticuerpo, cambiando la composición de glucosilación (patrón de glucosilación), o la magnitud de la glucosilación.

Anticuerpos pueden glucosilarse en posiciones conservadas (N-enlazadas u O-enlazadas) en sus regiones constantes (Hse et al (1997) J. Biol. Chem. 272:9062-9070; Jefferis and Lund, (1997) Chem. Immunol. 65:111-128; Wright and Morrison, (1997) TibTECH 15:26-32). Las cadenas laterales de oligosacárido de las inmunoglobulinas afectan la función de la proteína (Boyd et al (1996) Mol. Immunol. 32:1311-1318; Wittwe and Howard, (1990) Biochem. 29:4175-4180), y la interacción intramolecular entre porciones de la glucoproteína, que pueden afectar la conformación y presentan superficie tridimensional de la glucoproteína (Hefferis and Lund, *supra*; Wyss and Wagner (1996) Current Opin. Biotech. 7:409-416). Asimismo, los oligosacáridos pueden servir para direccionar una glucoproteína dada a ciertas moléculas, basándose en estructuras de reconocimiento específico (Malhotra et al (1995) Nature Med. 1:237-243; Umana et al (1999) Nature Biotech. 17:176-180). El retiro de los oligosacáridos puede optimizar la unión de antígeno y otras propiedades del anticuerpo (Boyd et al (1996) Mol. Immunol. 32:1311-1318).

Factores que afectan la glucosilación durante la producción recombinante de anticuerpos incluyen el modo de crecimiento, la formulación del medio, la densidad del cultivo, la oxigenación, el pH, los esquemas de purificación y similares (US 5047335; US 5510261; US 5278299). La glucosilación, o ciertos tipos de glucosilación, pueden retirarse enzimáticamente de la glucoproteína, por ejemplo usando endoglucosidasa H (Endo H). Adicionalmente, la célula hospedera recombinante puede manipularse genéticamente, *por ejemplo* hacerse defectuosa en procesar ciertos tipos de polisacáridos. Éstas y otras técnicas similares son bien conocidas en el arte.

La estructura de glucosilación de anticuerpos puede analizarse fácilmente por técnicas convencionales de análisis de carbohidratos, incluida cromotografía de lectina, NMR, espectrometría de masa, HPLC, GPC, análisis compuesto de monosacáridos, digestión enzimática secuencial, y HPAEC-PAD, que utiliza cromatografía de intercambio

aniónico con pH alto para separar oligosacáridos basado en carga. Se conocen también métodos para liberar oligosacáridos para propósitos analíticos, e incluyen, sin limitación, tratamiento enzimático (realizado comúnmente usando péptido-N-glucosidasa F/endo-β-galactosidasa), eliminación usando un ambiente alcalino áspero para liberar estructuras principalmente O-enlazadas, y métodos químicos usando hidrazida anhidra para liberar oligosacáridos N- y O-enlazados.

FRACCIONES DE MEDICAMENTO MAITANSINOIDE

Los compuestos de maitansina inhiben la proliferación celular impidiendo la formación de microtúbulos durante la mitosis, mediante la inhibición de polimerización de la proteína microtubulina, tubulina (Remillard et al (1975) Science 189:1002-1005; US 5208020). La maitansina y los maitansinoides son altamente citotóxicos, pero su empleo en terapia de cáncer se ha limitado en gran medida por sus graves efectos secundarios sistémicos, atribuidos principalmente a su escasa selectividad para tumores. Estudios clínicos con maitansina se habían descontinuado debido a serios efectos secundarios sobre el sistema nervioso central y el sistema gastrointestinal (Issel et al (1978) Can. Treatment. Rev. 5:199-207.

Las fracciones de medicamento maitansinoide son fracciones de medicamento atractivas en conjugados anticuerpo-medicamento, ya que son: (i) relativamente accesibles para preparar por fermentación o modificación química, derivación de productos de fermentación, (ii) susceptibles de derivación con grupos funcionales apropiados para conjugación mediante enlazadores no disulfuro con anticuerpos, (iii) estables en el plasma, y (iv) efectivas contra una variedad de líneas celulares tumorales.

Compuestos maitansinoides apropiados para uso como fracciones de medicamento de maitansina son bien conocidos en el arte, y pueden aislarse de fuentes naturales de acuerdo con métodos conocidos, producidos usando técnicas de ingeniería genética (véase Yu et al (2002) PNAS 99:7968-

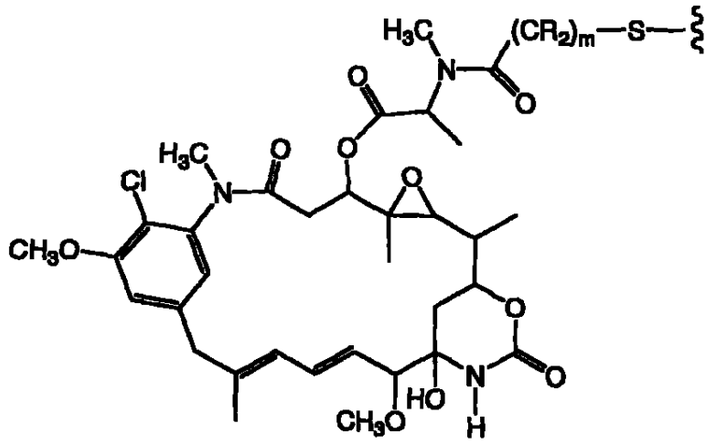
7973), o maitansinol y análogos de maitansinol preparados sintéticamente de acuerdo con métodos conocidos.

Fracciones ejemplares de medicamento maitansinoide incluyen aquellas con un anillo aromático modificado, tal como: C-19-decloro (US 4256746) (preparado por reducción de hidruro de litio y aluminio de ansamitocina P2); C-20-hidroxi (ó C-20-demetil) +/-C-19-decloro (Patentes de los Estados Unidos Nos. 4361650 y 4307016) (preparado por demetilación usando *Streptomyces* o *Actinomyces* o decloración usando LAH); y C-20-demetoxi, C-20-aciloxi (-OCOR), +/- decloro (US 4294757) (preparados por acilación usando cloruros de acilo), y aquellas con modificaciones en otras posiciones.

Fracciones ejemplares de medicamento maitansinoide incluyen asimismo aquellas con modificaciones tales como: C-9-SH, preparado por la reacción de maitansinol con H_2S ó P_2S_5 (US 4424219); C-14-alcoximetil(demetoxi/CH_2 OR)(US 4331598); C-14-hidroximetil o aciloximetil (CH_2OH ó CH_2OAc) preparados de Nocardia (US 4450254); C-15-hidroxi/aciloxi, preparado por la conversión de maitansinol por Streptomyces (US 4364866); C-15-metoxi, aislado de Trewia nudlflora (US 4313946 y US 4315929); C-18-N-demetil, preparado por la demetilación de maitansinol por Streptomyces (US 4362663 y US 4322348); y 4,5-deoxi, preparado por reducción de tricloruro de titanio/LAH de maitansinol (US 4371533).

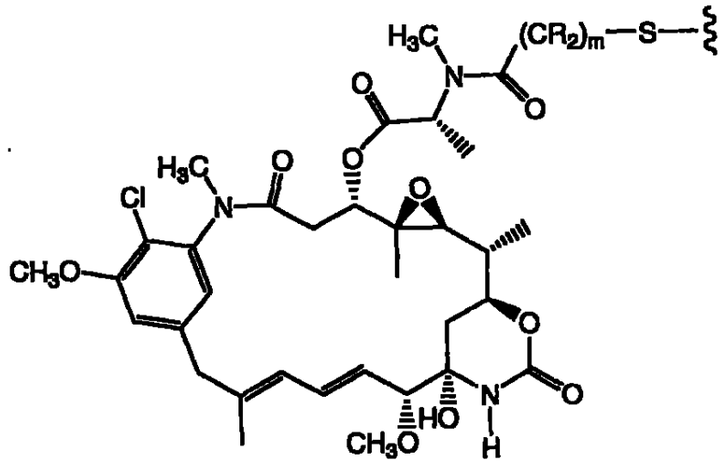
Muchas posiciones en compuestos de maitansina se conocen por ser útiles como la posición de ligamiento, dependiendo del tipo de enlace. Por ejemplo, para formar un ligamiento éster, la posición C-3 con un grupo hidroxilo, la posición C-14 modificada con hidroximetilo, la posición C-15 modificada con un grupo hidroxilo y la posición C-20 con un grupo hidroxilo son todas apropiadas.

Fracciones de medicamento maitansinoide (D) incluyen aquellas que tienen la estructura:



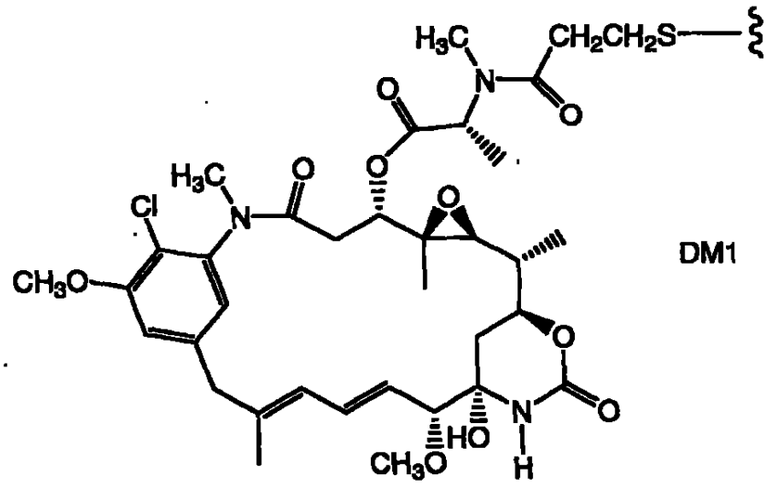
donde la línea ondulada indica la unión covalente del átomo de azufre de D a un enlazador (L) de un conjugado anticuerpo-medicamento (ADC). R puede ser independientemente H ó un C_1-C_6 alquilo. La cadena alquileno, que une el grupo amida al átomo de azufre, puede ser metanilo, etanilo, o propilo, *es decir*, m es 1, 2, ó 3 (US 633410, 5208020, Chan et al (1992) Cancer Res. 52:127-131; Liu et al (1996) Proc. Natl. Acad. Sci 93:8618-8623).

Los estereoisómeros de la fracción de medicamento maitansinoide se contemplan para los compuestos de la invención, *es decir*, cualquier combinación de configuraciones R y S en los carbonos quirales de D. En una incorporación, la fracción de medicamento maitansinoide (D) tendrá la siguiente estereoquímica:



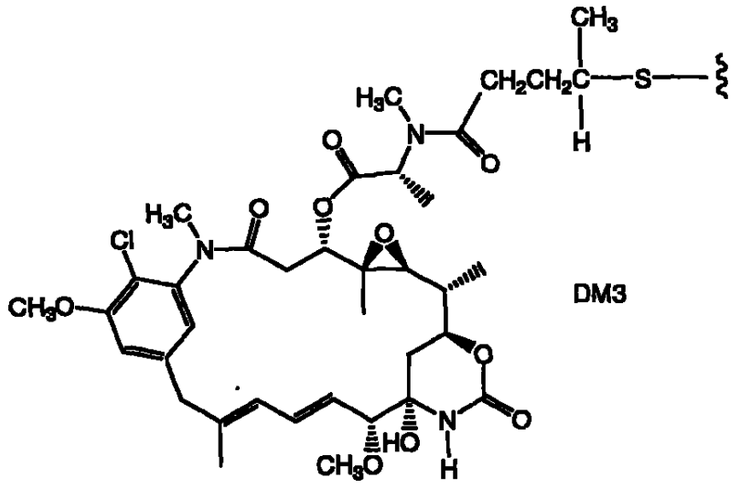
Incorporaciones de D incluyen:

DM1 ($N^{2'}$-deacetil-$N^{2'}$-(3-mercapto-1-oxopropil)-maitansina) donde $(CR_2)_m$ = CH_2CH_2;



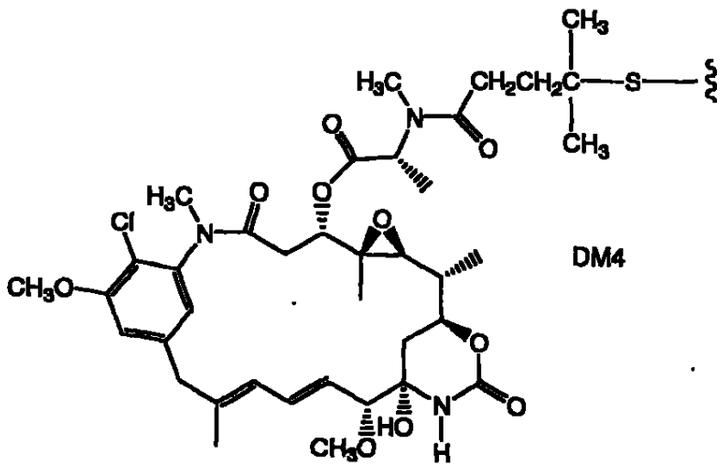
DM1

DM3 ($N^{2'}$-deacetil-$N^{2'}$(4-mercapto-1-oxopentil)-maitansina) donde $(CR_2)_m$ = $CH_2CH_2CH(CH_3)$;



DM3

DM4 ($N^{2'}$-deacetil-$N^{2'}$-(4-metil-4-mercapto-1-oxopentil)-maitansina) donde $(CR_2)_m$ = $CH_2CH_2C(CH_3)_2$:



DM4

La inhibición estérica, conferida por grupos alquílicos tales como los grupos metilo en el carbono adyacente al átomo de azufre de DM3 y DM4, puede afectar la velocidad de segmentación intracelular del ADC (US 2004/0235840 A1). Por lo tanto, la unidad alquilo variable $(CR_2)_m$ puede afectar la potencia, eficacia, y seguridad/toxicidad *in vitro* e *in vivo*.

ENLAZADORES

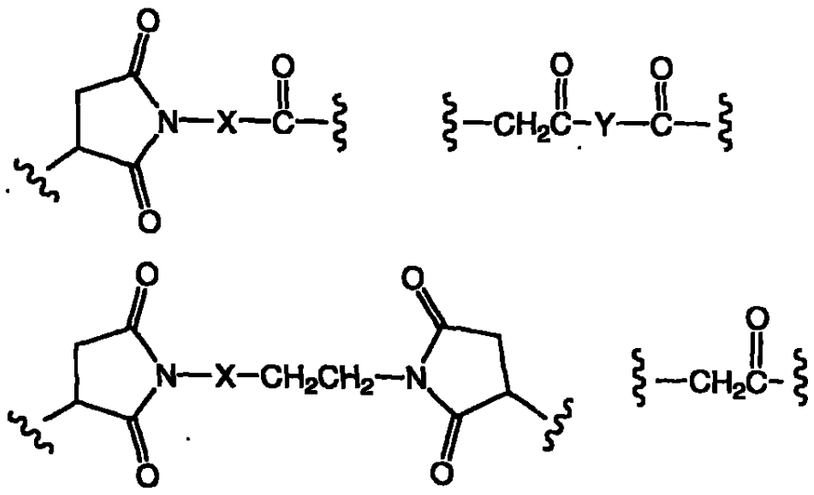
El enlazador, L, une el anticuerpo a una fracción de medicamento mediante enlace(s) covalente, que no comprende un grupo disulfuro. El enlazador es una fracción bifuncional o multifuncional, la cual puede usarse para conectar una o más fracciones de medicamento (D) y una unidad anticuerpo (Ab) para formar conjugados anticuerpo-medicamento (ADC) de Fórmula I. Conjugados anticuerpo-medicamento (ADC) pueden prepararse convenientemente usando un enlazador con funcionalidad reactiva para unión al medicamento y al anticuerpo. Un tiol de cisteína, o una amina, *por ejemplo* una cadena lateral de aminoácido de N-término tal como lisina, del anticuerpo (Ab) puede formar un enlace con un grupo funcional de un reactivo enlazador, fracción de medicamento o reactivo medicamento-enlazador.

Preferiblemente los enlazadores son estables extracelularmente. Antes del transporte o suministro a una célula, el conjugado anticuerpo-medicamento (ADC) es preferiblemente estable y permanece intacto, *es decir*, el anticuerpo permanece conectado a la fracción de medicamento.

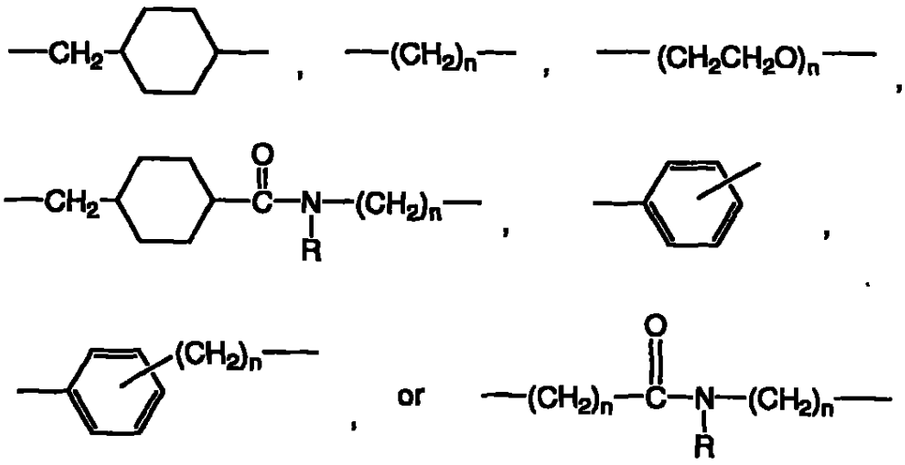
Los enlazadores son estables fuera de la célula diana y pueden segmentarse con alguna velocidad eficaz dentro de la célula. Un enlazador efectivo: (i) mantendrá las propiedades de unión específica del anticuerpo; (ii) permitirá el suministro intracelular del conjugado a la fracción de medicamento; (iii) permanecerá estable e intacto, *es decir*, no segmentado, hasta cuando el conjugado se haya dispensado o transportado a su sitio seleccionado; y (iv) mantendrá un efecto citolítico, citotóxico, o un efecto citostático de la fracción de medicamento maitansinoide. La estabilidad del ADC puede medirse por técnicas analíticas estándar, tales como espectroscopia de masa, HPLC, y la técnica de separación/análisis LC/MS.

La unión covalente del anticuerpo y la fracción de medicamento requiere que el enlazador tenga dos grupos funcionales reactivos, *es decir*, bivalencia en sentido reactivo. Se conocen reactivos de enlazador bivalente, que son útiles para unir dos o más fracciones funcionales o biológicamente activas, tales como péptidos, ácidos nucleicos, medicamentos, toxinas, anticuerpos, haptenos, y grupos reporteros, y se han descrito métodos para preparar sus conjugados resultantes (Hermanson, G.T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p234-242).

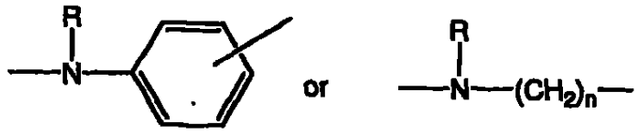
Los enlazadores pueden tener estructuras seleccionadas de:



donde las líneas onduladas indican las uniones covalentes a Ab y D en cualquiera de las dos orientaciones. X puede tener las estructuras, en cualquiera de las dos orientaciones:

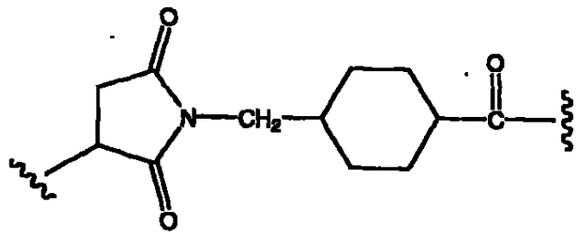


donde R es independientemente H ó C_1-C_6 alquilo; y n es 1 a 12. Y puede tener las estructuras, en cualquiera de las dos orientaciones:

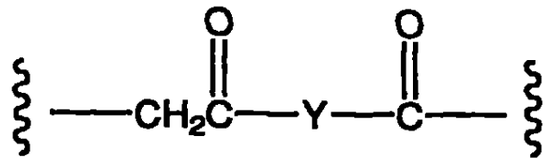


donde R es independientemente H ó C_1-C_6 alquilo; y n es 1 a 12.

Por ejemplo, el enlazador puede tener la estructura, designada como SMCC:

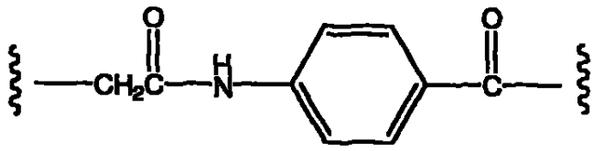


En otra incorporación, el enlazador (1) tiene la estructura:

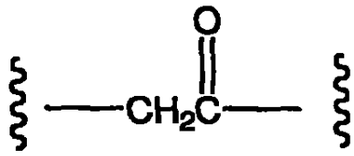


donde las líneas onduladas indican las uniones covalentes a Ab y D en cualquiera de las dos orientaciones.

Por ejemplo, el enlazador puede tener la estructura, designada como SIAB:



En otra incorporación, el enlazador (L) tiene la estructura:



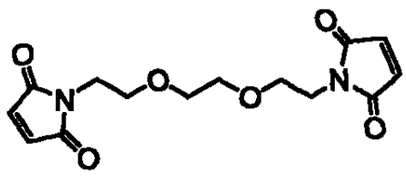
En otra incorporación, el enlazador puede sustituirse con grupos que modulan solubilidad o reactividad. Por ejemplo, un sustituyente sulfonato puede aumentar la solubilidad en agua del reactivo y facilitar la reacción de acoplamiento del reactivo enlazador con el anticuerpo de la fracción de medicamento, o facilitar la reacción de acoplomiento de Ab-L con D, o D-L con Ab, dependiendo de la ruta sintética empleada para preparar el ADC.

En otra incorporación, un enlazador posee un grupo funcional reactivo, que tiene un grupo nucleófilo que es reactivo a un grupo electrófilo presente en un anticuerpo. Grupos electrófilos útiles en un anticuerpo incluyen, pero no se limitan a, aldehído y grupos acetona carbonilo. El heteroátomo de un grupo nucleófilo de un enlazador puede reaccionar con un grupo electrófilo en un anticuerpo y formar un enlace covalente a una unidad anticuerpo. Grupos nucleófilos útiles en un enlazador incluyen, pero no se limitan a, hidrazida, oxime, amino, hidrazina, tiosemicarbazona, carboxilato de hidrazina, y arilhidrazida. El grupo electrófilo en un anticuerpo provee un sitio conveniente para unión a un enlazador.

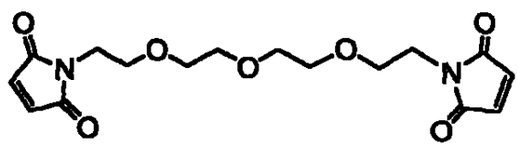
Los enlazadores pueden ser peptídicos, comprendiendo una o más unidades de aminoácidos. Reactivos de enlazador peptídico pueden prepararse por métodos de síntesis de fase sólida o fase líquida (E. Schröder and K. Lübke, *The Peptides*, volume 1, pp 76-136 (1965) Academic Press) que son bien conocidos en el campo de la química de péptidos, incluida

química t-BOC (Geiser et al "Automation of solid-phase peptide synthesis" in *Macmmolecular Sequencing and Synthesis*, Alan R. Liss, Inc., 1988, pp. 199-218) y química Fmoc/HBTU (Fields, G. and Noble, R. (1990) "Solid phase peptide synthesis utilizing 9-fluoroenylmethoxycarbonyl amino acids", Int. J. Peptide Protein Res. 35:161-214), on an automated synthesizer such as the Rainin Symphony Peptide Synthesizer (Protein Technologies, Inc., Tucson, AZ), or Model 433 (Applied Biosystems, Foster City, CA).

Los compuestos contemplan expresamente, pero no se limitan a, ADC preparado con reactivos de enlazador cruzado: BMPEO, 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 (succinimidil-(4-vinilsulfona)benzoato), e incluidos reactivos de bis-maleimida: DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, y $BM(PEO)_4$, que se encuentran disponibles comercialmente de 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. Véase Páginas 467-498, 2003-2004 Applications Handbook and Catalog. Los reactivos de bis-maleimida permiten la unión de un grupo tiol libre de un residuo cisteína de un anticuerpo a una fracción de medicamento con contenido de tiol, marcador, o intermedio de enlazador, en una forma secuencial o concurrente. Otros grupos funcionales aparte de maleimida, que son reactivos con un grupo tiol de un anticuerpo, fracción de medicamento maitansinoide, o intermedio enlazador, incluyen yodoacetamida, bromoacetamida, vinilpiridina, disulfuro, piridil disulfuro, isocianato, e isotiocianato.

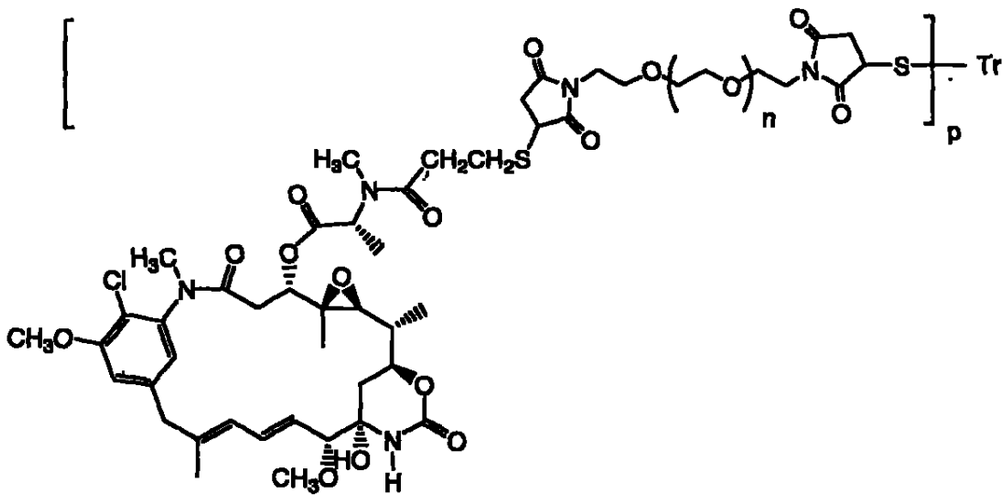


$BM(PEO)_3$



$BM(PEO)_4$

Conjugados ejemplares anticuerpo-medicamento, donde DM1 se enlaza mediante un enlazador BMPEO a un grupo tiol de trastuzumab, tienen la estructura:



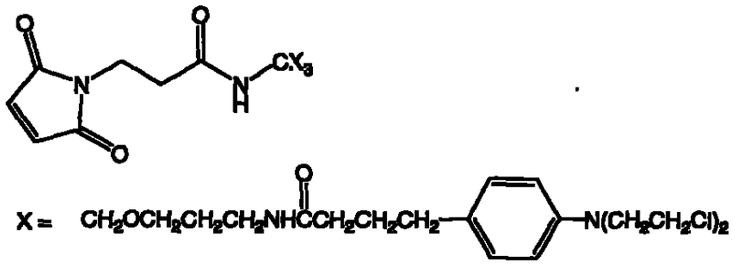
donde Tr es trastuzumab; n es 0, 1, ó 2; y p es 1, 2, 3, ó 4.

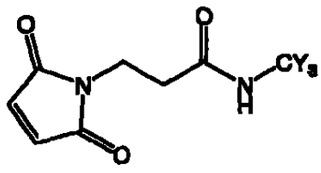
Reactivos de enlazador útiles pueden obtenerse también a través de otras fuentes comerciales, tales como Molecular Biosciences Inc.(Boulder, CO), o sintetizarse de acuerdo con procedimientos descritos en Toki et al (2002) J. Org. Chem. 67:1866-1872; US 6214345 to Firestone et al; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; y WO 04/032828..

El enlazador puede ser un enlazador tipo dendrítico para unión covalente de más de una fracción de medicamento por medio de una fracción de enlazador multifuncional, ramificante, a un anticuerpo (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768; King et al (2002) Tetrahedron Letters 43: 1987-1990). Los enlazadores dendríticos pueden aumentar la relación molar de medicamento a anticuerpo, *es decir*, carga, que se relaciona con la potencia del ADC. Por consiguiente, donde un anticuerpo lleva únicamente un grupo tiol cisteína reactivo, una multitud de fracciones de medicamento puede unirse mediante un enlazador dendrítico.

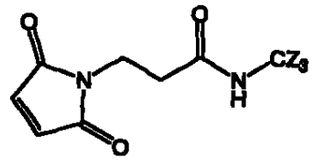
Las siguientes incorporaciones ejemplares de reactivos de enlazador dendrítico permiten conjugar hasta nueve reactivos de fracción de

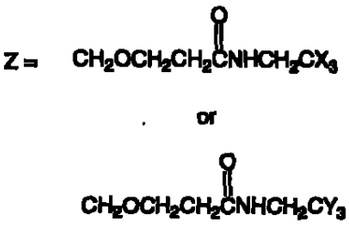
medicamento nucleófilo, mediante reacción con los grupos funcionales de mostaza cloroetil nitrogenada:











CARGA DE MEDICAMENTO

La carga de medicamento se representa por **p** en una molécula de Fórmula **I**, el número promedio de medicamentos maitansinoides por anticuerpo. La carga de medicamento puede variar de 1 a 8 medicamentos (D) por anticuerpo (Ab), *ies decir*, donde 1, 2, 3, 4, 5, 6, 7, y 8 fracciones de medicamento se unen covalentemente al anticuerpo. Composiciones de ADC de Fórmula **I** incluyen colecciones de anticuerpos conjugados con un rango de medicamentos, de 1 a 8. El número promedio de medicamentos por anticuerpo en preparaciones de ADC de reacciones de conjugación puede caracterizarse por medios convencionales tales como espectroscopia de masa, análisis ELISA, electroforesis, y HPLC. Igualmente puede determinarse la distribución cuantitativa de ADC en términos de **p**.

Mediante ELISA puede determinarse el valor promediado de p en una preparación particular de ADC (Hamblett et al (2004) Clinical Cancer Res. 10:7063-7070; Sanderson et al (2005) Clinical Cancer Res. 11:843-852). No obstante, la distribución de los valores de p (medicamento) no es discernible por la unión anticuerpo-antígeno y la limitación de detección de ELISA. Asimismo, el ensayo ELISA para detección de conjugados anticuerpo-medicamento no determina dónde las fracciones de medicamento se unen al anticuerpo, tales como los fragmentos de cadena pesada o cadena ligera, o los residuos de aminoácidos particulares. En algunos casos, la separación, purificación y caracterización de ADC homogéneos, donde p es un cierto valor de ADC con otras cargas de medicamento, pueden lograrse por medios tales como HPLC de fase reversa o electroforesis.

Para algunos conjugados anticuerpo-medicamento, p puede estar limitado por el número de sitios de unión en el anticuerpo. Por ejemplo, donde la unión es un tiol cisteína, como en las anteriores incorporaciones ejemplares, un anticuerpo puede tener únicamente uno o varios grupos tiol, cisteína, o puede tener únicamente uno o varios grupos tiol suficientemente reactivos, mediante los cuales puede unirse un enlazador. Una mayor carga de medicamento, *por ejemplo* p>5, puede causar agregación, insolubilidad, toxicidad, o pérdida de permeabilidad celular de ciertos conjugados anticuerpo-medicamento.

Típicamente, menos que el máximo teórico de fracciones de medicamento se conjugan con un anticuerpo durante una reacción de conjugación. Un anticuerpo puede contiene, por ejemplo, muchos residuos de lisina que no reaccionan con el intermedio medicamento-enlazador (D-L) o reactivo enlazador. Únicamente los grupos lisina más reactivos pueden reaccionar con un reactivo enlazador reactivo con amina. Asimismo, sólo los grupos tiol cistaína más reactivos pueden reaccionar con un reactivo enlazador que reacciona con tiol. Generalmente, los anticuerpos no contienen muchos, si lo hay, grupos tiol cisteína reactivos y libres, que puedan enlazarse con una fracción de medicamento. La mayoría de los

residuos tiol cisteína en los anticuerpos de los compuestos existen como puentes disulfuro y tienen que reducirse con una agente reductor tal como ditiotreitol (DTT) o TCEP, bajo condiciones de reducción parcial o total. Adicionalmente, el anticuerpo tiene que someterse a condiciones de desnaturalización para revelar grupos nucleófilos tales como lisina o cisteína. La carga (relación medicamento/anticuerpo) de un ADC puede controlarse en varias manera diferentes, incluyendo: (i) limitar el exceso molar del intermedio medicamento-enlazante (D-L) o reactivo enlazador en relación con el anticuerpo, (ii) limitar el tiempo de reacción de conjugación o temperatura, y (iii) condiciones de reducción limitante o parcial para la modificación tiol cisteína.

Donde más de un grupo nucleófilo o electrófilo del anticuerpo reacciona con un intermedio medicamento-enlazador, o reactivo enlazador seguido por reactivo de fracción de medicamento, entonces el producto resultante es una mezcla de compuestos ADC con una distribución de fracciones de medicamento unidas a un anticuerpo, *por ejemplo* 1, 2, 3, etc. Métodos de cromatografía de líquidos, tales como fase reversa polimérica (PLRP) e interacción hidrófoba (HIC), pueden separar compuestos en la mezcla por valor de carga de medicamento. Preparaciones de ADC con un valor de carga de medicamento único (p) pueden aislarse ("Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate", Hamblett, K.J., et al, Abstract No. 624, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; "Controlling the Location of Drug Attachment in Antibody-Drug Conjugates", Alley, S.C., et al, Abstract No. 627, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). Sin embargo, estos ADC de valor de carga única pueden ser aún mezclas heterogéneas, porque las fracciones de medicamento pueden unirse, mediante el enlazador, en diferentes sitios en el anticuerpo.

PREPARACIÓN DE CONJUGADOS ANTICUERPO-MEDICAMENTO

El ADC de Fórmula I puede prepararse por varias rutas, empleando reacciones de química orgánica, condiciones, y reactivos conocidos por los expertos en el arte, incluidas: (1) reacción de un grupo nucleófilo o un grupo electrófilo de un anticuerpo con un reactivo enlazador bivalente, para formar el intermedio anticuerpo-enlazador Ab-L, mediante un enlace covalente, seguida por reacción con una fracción de medicamento activada D; y (2) reacción de un grupo nucleófilo o un grupo electrófilo de una fracción de medicamento con un reactivo enlazador, para formar el intermedio medicamento-enlazador D-L, mediante un enlace covalente, seguida por reacción con el grupo nucleófilo o un grupo electrófilo de un anticuerpo. Métodos de conjugación (1) y (2) pueden emplearse con una variedad de anticuerpos, fracciones de medicamento, y enlazadores para preparar los conjugados anticuerpo-medicamento de Fórmula I.

Grupos nucleófilos en anticuerpos incluyen, pero no se limitan a: (i) grupos amina N-terminal, (ii) grupos amina de cadena lateral, *por ejemplo*, lisina, (iii) grupos tiol de cadena lateral, *por ejemplo*, cisteína, y (iv) grupos azúcar hidroxilo o amino donde el anticuerpo es glucosilado. Grupos amina, tiol, e hidroxilo son nucleófilos y capaz de reaccionar para formar enlaces covalentes con grupo electrófilos en fracciones de enlazador y reactivos de enlazador incluidos: (i) ésteres activos tales como ésteres NHS, ésteres HOBt, haloformiatos, y haluros ácidos; (ii) haluros alquílicos y bencílicos tales como haloacetamidas; (iii) aldehídos, acetonas, carboxilo, y grupos maleimida. Ciertos anticuerpos tienen disulfuros de intercadena reducibles, *es decir*, puentes cisteína. Anticuerpos pueden hacerse reactivos para conjugación con reactivos enlazadores por tratamiento con un agente reductor tal como DTT (reactivo de Cleland, ditiotreitol) o TCEP (tris(2-carboxietil)fosfina clorhidrato; Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA). Por consiguiente, cada puente disulfuro cisteína formará, teóricamente, dos nucleófilos tiol reactivos. Grupos nucleófilos adicionales pueden introducirse en anticuerpos mediante

la reacción de lisinas con 2-iminotiolano (reactivo de Traut) resultante en la conversión de una amina en un tiol.

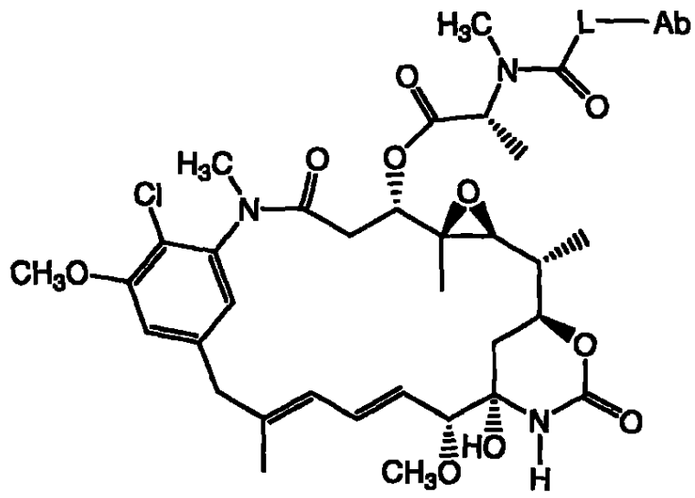
Conjugados anticuerpo-medicamento pueden producirse asimismo por modificación del anticuerpo para introducir fracciones electrófilas, que pueden reaccioanr con sustituyentes nucleófilos en el reactivo enlazador o medicamento. Los azúcares de anticuerpos glucosilados pueden oxidarse, *por ejemplo* con reactivos de oxidación con periodato, para formar aldehído o grupos acetona, los cuales pueden reaccionar con el grupo amina de reactores de enlazador o fracciones de medicamento. Los grupos básicos de imina Schiff resultantes pueden formar un enlazamiento estable, o pueden reducirse, *por ejemplo* por reactivos borohidruro para formar enlazamientos de amina estables. En una incorporación, la reacción de la porción carbohidrato de un anticuerpo glucosilado con galactosa oxidasa o meta-periodato de sodio puede proporcionar grupos carbonilo (aldehído y acetona) en la proteína que puede reaccionar con grupos apropiados en el medicamento (Hermanson, G. T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p 234-242). En otra incorporación, proteínas que contienen residuos de serina o treonina N-terminal pueden reaccionar con meta-periodato de sodio, resultante en la producción de un aldehído en lugar del primer aminoácido (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Tal aldehído puede hacerse reaccionar con una fracción de medicamento o nucleófio enlazador.

Análogamente, grupos nucleófilos en una fracción de medicamento incluyen, pero no se limitan a: amina, tiol, hidroxilo, hidrazida, oxime, hidrazina, tiosemicarbazona, hidrazina carboxilato, y grupos arilhidrazida capaces de reaccionar para formar enlaces covalentes con grupos electrófilos en fracciones de enlazador y reactivos de enlazador incluidos: (i) ésteres activos tales como ésteres NHS, ésteres HOBt, haloformiatos, y haluros ácidos; (ii) haluros alquílicos y bencílicos tales como haloacetamidas; (iii) aldehídos, acetonas, carboxilo, y grupos maleimida.

Maitansina puede, por ejemplo, convertirse en May-$SSCH_3$, que puede reducirse al tiol libre, May-SH, y hacerse reaccionar con un

anticuerpo modificado (Chari et al (1992) Cancer Research 52:127-131) para generar un inmunoconjugado maitansinoide-anticuerpo con un enlace disulfuro. Se han divulgado conjugados anticuerpo-maitansinoide con enlazadores disulfuro (WO 04/016801; US 6884874; US 2004/039176 A1; WO 03/068144; US 2004/001838 A1; US 6441163; US 5208020; US 5416064; WO 01/024763). El enlazador disulfuro SPP se construye con reactivo de enlazador. N-succinimidil 4-(2-piridiltio) pentanoato. Conjugados anticuerpo-SPP-DM1 se representan por la estructura:

Conjugados anticuerpo-medicamento de enlazador disulfuro (S-S) se ensayaron para efectos de comparación con el ADC de enlazador no disulfuro de la invención. Trastuzumab-SPP-DM1 se preparó de acuerdo con Ejemplo3 (Ranson, M. and Sliwkowski M. (2002) Oncology 63(suppl 1):17-24). También se ensayaron conjugados anticuerpo-medicamento de disulfuro: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM3, y trastuzumab-SPP-DM4, y tienen las siguientes estructuras:

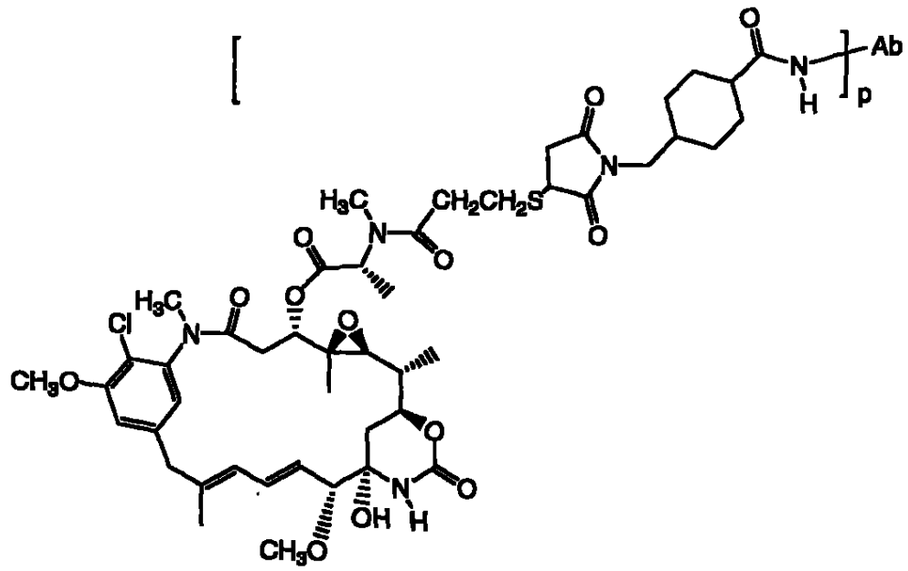


L—— Ab = CH_2CH_2-S-S-CH_2CH_2CHNH——Tr Trastuzumab-SPDP-DM1

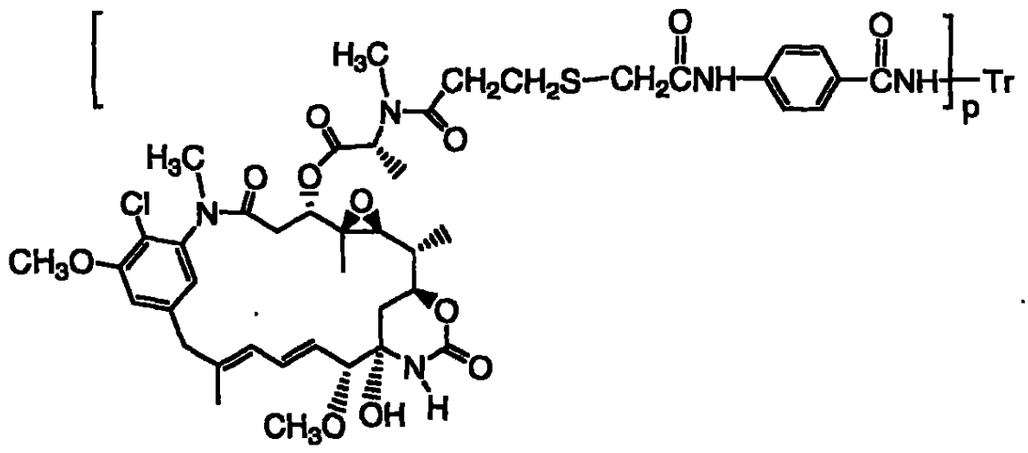
L—— Ab = $CH_2CH(CH_3)$-S-S-$CH(CH_3)CH_2CH_2CHNH$–Tr Trastuzumab-SPP-DM3

L——Ab = $CH_2C(CH_3)_2$-S-S-$CH(CH_3)CH_2CH_2CHNH$–Tr Trastuzumab-SPP-DM4

ADC de la invención incluyen enlazadores SMCC y la fracción de medicamento maitansonoide DM1, representada como Ab-SMCC-DM1:



Una incorporación de Ab-SMCC-DM1 es trastuzumab-SMCC-DM1 donde **p** es 1, 2, 3, ó 4 (Ab = trastuzumab, Tr, WO 2005/037992). Otra incorporación de un ADC es trastuzumab-SIAB-DM1 (trastuzumab = Tr), que tiene la estructura:



CRIBADO DE CONJUGADOS ANTICUERPO-MEDICAMENTO (ADC) DIRIGIDOS CONTRA ANTÍGENOS ASOCIADOS CON TUMORES Y RECEPTORES DE SUPERFICIE CELULAR

Animales transgénicos y líneas celulares son particularmente útiles en cribar conjugados anticuerpo-medicamento (ADC) que tienen potencial

como tratamiento terapéuticos o profilácticos de enfermedades o trastornos, que involucran la sobreexpresión de antígenos asociados con tumor y receptores de superficie celular, *por ejemplo*, HER2 (US 6632979). El cribado de un ADC útil puede implicar administrar ADC candidato sobre un rango de dosis al animal transgénico, y analizar en varios puntos de tiempo el efecto(s) del ADC sobre la enfermedad o trastorno, que se está evaluando. Alternativa, o adicionalmente, el medicamento puede administrarse previa o simultáneamente con la exposición a un inductor de la enfermedad, si es el caso. ADC candidato puede cribarse en serie e individualmente, o en paralelo bajo formato de cribado medio o de alto rendimiento. La velocidad, a la cual puede cribarse el ADC respecto a su utilidad para los tratamientos terapéuticos o profilácticos de enfermedades o trastornos, se limita únicamente por la velocidad de síntesis o metodología de cribado, incluida la detección/medición/análisis de datos.

Una incorporación es un método de cribado que comprende (a) transplantar células de una línea celular estable de cáncer de mama a un animal no humano, (b) administrar un medicamento ADC candidato al animal no humano y (c) determinar la capacidad del candidato para inhibir la formación de tumores a partir de la línea celular transplantada. La invención concierne igualmente a un método de cribar ADC candidatos para el tratamiento de una enfermedad o trastorno caracterizado por la sobreexpresión de una proteína de receptor, que comprende (a) contactar células de una línea celular estable de cáncer de mama con un medicamento candidato y (b) evaluar la capacidad del ADC candidato para inhibir el crecimiento de la línea celular estable.

Una incorporación es un método de cribado que comprende (a) contactar células de una línea celular estable de cáncer de mama con un medicamento ADC candidato y (b) evaluar la capacidad del ADC candidato de bloquear la activación de ligando de HER2. Otra incorporación es evaluar la capacidad del ADC candidato de bloquear la unión de herregulina. En otra incorporación se evalúa la capacidad del ADC candidato de bloquear fosforilación de tirosina estimulada por ligando.

Otra incorporación es un método de cribado que comprende (a) contactar células de una línea celular estable de cáncer de mama con un medicamento ADC candidato y (b) evaluar la capacidad del ADC candidato de inducir muerte celular. En una incorporación se evalúa la capacidad del ADC candidato de inducir apoptosis.

Otra incorporación es un método de cribado que comprende (a) administrar un medicamento ADC candidato a un mamífero transgénico no humano, que sobreexpresa, *por ejemplo*, en sus células de glándula mamaria, una proteína humana nativa, *por ejemplo*, HER2 o un fragmento de la misma, en donde tal mamífero transgénico ha integrado establemente en su genoma una secuencia de ácidos nucleicos que codifica la proteína humana nativa o un fragmento de la misma, que tiene la actividad biológica de la proteína humana nativa, enlazada operativamente a secuencias reguladoras transcripcionales que dirigen su expresión, y desarrolla un tumor, *por ejemplo*, un tumor mamario, que no responde o responde a medias a tratamiento de anticuerpo, *por ejemplo*, anti-HER2, o a un mamífero no humano que porta un tumor transplantado de dicho mamífero no humano transgénico; y (b) evaluar el efecto del ADC candidato sobre la enfermedad o trastorno específicos. Sin limitaciones, la enfermedad o trastorno puede ser un cáncer que sobreexpresa HER2, tal como cáncer de mama, ovarios, estómago, endometrio, glandula salival, pulmón, riñón, colon, tiroides, páncreas y vejiga. El cáncer puede ser cáncer de mama que expresa HER2 en por lo menos alrededor de 500.000 copias por célula, o por lo menos alrededor de 2.000.000 copias por célula. Los medicamentos ADC candidatos pueden evaluarse, por ejemplo, por su capacidad de inducir muerte celular y/o apoptosis, usando métodos de análisis bien conocidos en el arte y descritos más adelante.

En una incorporación, ADC candidatos se criban administrándolos al animal transgénico en un rango de dosis, y evaluando la respuesta fisiológica del animal a los compuestos en el transcurso del tiempo. La administración puede ser, o mediante inyección apropiada, dependiendo de la índole químicadel compuesto que se está evaluando. En algunos casos,

puede ser apropiado administra el compuesto en conjunción con co-factores que mejorarían la eficacia del compuesto. Si líneas celulares derivadas de los animales transgénicos sujetos se usan para cribar los compuestos útiles en tratar varios trastornos asociados con la sobreexpresión de ciertas proteínas de antígenos asociadas con tumores o receptores de superficie celular, *por ejemplo*, sobreexpresión de HER2, los compuestos de prueba se añaden al medio de cultivo celular en un tiempo apropiado, y se evalúa la respuesta celular al compuesto durante el tiempo usando los análisis bioquímicos y/o histológicos apropiados. En algunos casos, puede ser apropiado aplicar el compuesto de interés al medio de cultivo en conjunción con co-factores que mejorarían la eficacia del compuesto.

Por consiguiente, la presente invención provee análisis para identificar ADC, que específicamente localicen y unan la proteína HER2 sobreexpresada, cuya presencia se correlaciona con función celular anormal, y en la patogénesis de proliferación celular y/o diferenciación de glándula mamaria, que casualmente se relaciona con el desarrollo de tumores de mama.

Para identificar un ADC que bloquea la activación de ligando de un receptor ErbB (*por ejemplo*, ErbB2), puede determinarse la capacidad del compuesto de bloquear la unión de ligando ErbB a células que expresan el receptor ErbB (ErbB2) (*epor ejemplo*, en conjugación con otro receptor ErbB, con el cual el receptor ErbB de interés forma un hetero-oligómero ErbB). Por ejemplo, células aisladas del animal transgénico, que sobreexpresan HER2 y transfectadas para expresar otro receptor ErbB (con el cual HER2 forma un hetero-oligómero) pueden incubarse, *por ejemplo*, cultivando, con el ADC y luego exponerse al ligando ErbB marcado. La capacidad del compuesto de bloquear la unión de ligando al receptor ErbB en el hetero-oligómero ErbB puede evaluarse entonces.

Por ejemplo, la inhibición de la unión de herregulina (HRG) a líneas de células de tumor de mama, que sobreexpresan HER2 y establecidas de los mamíferos transgénicos no humanos (*por ejemplo*, ratones) en esta solicitud, por el ADC candidato puede realizarse usando cultivos monocapa

sobre hielo en un formato de placa de 24 pocillos. Anticuerpos monoclonales anti-ErbB2 pueden agregarse a cada pocillo e incubarse por 30 minutos. A continuación puede agregarse rHRGβ$1_{177-224}$ ^{125}I-marcado (25.000 cpm), y la incubación puede continuarse por 4 a 16 horas. Pueden prepararse curvas de respuesta a la dosis y calcularse un valor IC_{50} para el compuesto de interés.

Alternativa, o adicionalmente, puede evaluarse la capacidad de un ADC de bloquear la fosforilación de tirosina estimulada por ligando ErbB de un receptor ErbB presente en un hetero-oligómero ErbB. Por ejemplo, líneas celulares, establecidas de los animales transgénicos en esta solicitud, pueden incubarse con un ADC de prueba y luego analizarse con respecto a la actividad de fosforilación de tirosina dependiente de ligando ErbB, usando un anticuerpo monoclonal anti-fosfotirosina (que se conjuga opcionalmente con un marcador detectable). El análisis de activación de receptor de cinasa descrito en US 5766863 es también utilizable para determinar la activación del receptor ErbB y el bloqueo de esa actividad por el compuesto.

En una incorporación, se puede cribar ADC que inhiban la estimulación HRG de fosforilación de tirosina p180 en células MCF7, esencialmente como se describe más adelante. Por ejemplo, una línea celular establecida de un animal transgénico HER2 puede esparcirse en placas de 24 pocillos, y el compuesto puede agregarse a cada pocillo e incubarse durante 30 minutos a temperatura de la sala; luego puede agregarse rHRGβ$1_{177-244}$ a cada pocillo a una concentración final de 0.2 nM, y la incubación puede continuarse durante aproximadamente 8 minutos. Puede aspirarse el medio de cada pocillo, y las reacciones pueden detenerse por la adición de 100 µl de tampón de muestra SDS (5% SDS, 25 mM DTT, y 25 mM Tris-HCl, pH 6.8). Cada muestra (25 µl) puede someterse a electroforesis en un gel de gradiente 4-12% (Novex) y luego transferirse electroforéticamente a una membrana de difluoruro de polivinilideno. Pueden desarrollarse inmunoblots de antifosfotirosina (a 1 µg/ml), y puede cuantificarse la intensidad de la franja reactiva predominante a M_r -180.000

mediante densitometría de reflexión. Un método alterno para evaluar la inhibición de la fosforilación de receptor es el análisis KIRA (activación de receptor de cinasa) (Sadick et al (1998) Jour, of Pharm. and Biomed. Anal. 1-9). Algunos de los anticuerpos monoclonales bien establecidos contra HER2, que son conocidos por inhibir la estimulación HRG de la fosforilación de tirosina p180, pueden utilizarse como control positivo en este análisis. Puede prepararse una curva de respuesta a la dosis para la inhibición de la estimulación HRG de fosforilación de tirosina p180, determinada por densitometría de reflexión, y calcularse una IC_{50} para el compuesto de interés.

Se pueden evaluar también los efectos inhibidores de crecimiento de un ADC de prueba en líneas celulares derivadas de un animal transgénico HER2 (Schaefer et al (1997) Oncogene 15:1385-1394). De acuerdo con este análisis, las células pueden tratarse con un compuesto de prueba en varias concentraciones durante 4 días y teñidas con cristal violeta o el tinte redox Alamar Blue. La incubación con el compuesto puede mostrar un efecto inhibidor de crecimiento en esta línea celular, similar al desplegado por el anticuerpo monoclonal 2C4 en células MDA-MB-175 (Schaefer et al., *supra*). En una incorporación adicional, HRG exógena no reversará significativamente esta inhibición.

Para identificar compuestos ADC inhibidores de crecimiento que específicamente localizan HER2, se pueden cribar ADC que inhiben el crecimiento de células cancerosas que sobreexpresan HER2, derivadas de animales transgénicos (US 5677171). De acuerdo con este análisis, células que sobreexpresan HER2 se cultivan en una mezcla 1:1 de medio F12 y DMEM suplementado con 10% de suero fetal bobino, glutamina y penicilina estreptomicina. Las células se esparcen a 20.000 células en una placa de cultivo de células de 35 mm (2 mls/35mm por placa) y el compuesto de prueba se añade en varias concentraciones. Después de seis días, se cuenta el número de células, comparado con células no tratadas usando un contador electrónico de células COULTER™. Aquellos ADC que inhiben el crecimiento celular en alrededor de 20-100% o alrededor de

50-100% pueden seleccionarse como compuestos inhibidores de crecimiento.

Para seleccionar ADC que inducen muerte celular, puede evaluarse la pérdida de integridad de la membrana como se indica, *por ejemplo*, PI, captación de trypan blue o 7AAD, con relación al control. El análisis de captación de PI utiliza células aisladas del tejido de tumor de mama de un animal transgénico. De acuerdo con este análisis, las células se cultivan en Dulbecco's Modified Eagle Medium (D-MEM): Ham's F-12 (50:50) suplementado con 10% FBS inactivado por calor (Hyclone) y 2 mM L-glutamina. Por consiguiente, el análisis se lleva a cabo en la ausencia de complemento de células efectoras inmunitarias. Las células se seleccionan con una densidad de 3 x 106 por placa, en placas de 100 x 20 mm y se deja que se adhieran de un día para otro. El medio se retira luego y reemplaza con medio fresco solo, o medio que contiene varias concentraciones del compuesto. Las células se incuban por un período de 3 días. Luego de cada tratamiento, se lavan las monocapas con PBS y se separan mediante tripsinización. A continuación las células se centrifugan a 1.200 rpm durante 5 minutos a 4 °C, el sedimento celular se resuspendió en 3 ml de tampón de unión Ca^{2+} frío (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) y repartió en alícuotas en tubos 12 x 75 mm tapados con un filtro de 35 mm (1 ml por tubo, 3 tubos por grupo de tratamiento) para el retiro de los grumos de células. Luego los tubos reciben PI (10 µg/ml). Las muestras pueden analizarse usando un citómetro de flujo FACSCAN™ y software FACSCONVERT™ CellQuest (Becton Dickinson). Aquellos compuestos, que inducen niveles estadísticamente significativos de muerte celular, determinados por captación PI, pueden seleccionarse como compuestos de inducción de muerte celular.

Con el fin de seleccionar compuestos que inducen apoptosis, se lleva a cabo un análisis de unión de annexina usando células establecidas del tejido de tumor de mama del animal transgénico. Las células se cultivan y siembran en placas, como se discute en el párrafo precedente. El medio se retira entonces y se reemplaza con medio fresco solo o medio que contiene

10 µg/ml del conjugado anticuerpo-medicamento (ADC). Luego de un período de incubación de tres días, las monocapas se lavaron con PBS y se separan por tripsinización. Las células se centrifugan entonces, se resuspenden en tampón de unión Ca^{2+} y se reparten en alícuotas en tubos, como se discute anteriormente, para el análisis de muerte celular. Seguidamente los tubos reciben annexina marcada (*por ejemplo*, annexina V-FITC) (1 µg/ml). Las muestras pueden analizarse usando un citómetro de flujo FACSCAN™ y software FACSCONVERT™ CellQuest (Becton Dickinson). Aquellos compuestos, que inducen niveles estadísticamente significativos de unión de anexina, en relación con el control, se seleccionan como compuestos de inducción de apoptosis.

ANÁLISIS DE PROLIFERACIÓN CELULAR IN VITRO

Generalmente, la actividad citotóxica o citostática del conjugado anticuerpo-medicamento (ADC) se mide: exponiendo células de mamífero, que tienen antígenos asociados con tumor o proteínas de receptor, al anticuerpo del ADC en un medio de cultivo de células; cultivar las células por un período de alrededor de 6 horas a alrededor de 5 días; y midiento la viabilidad celular. Análisis *in vitro* a base de células se utilizaron para medir la viabilidad, *es decir*, proliferación (IC_{50}), citotoxicidad (EC_{50}), e inducción de apoptosis (activación de caspasa) del ADC.

La potencia *in vitro* de conjugados anticuerpo-medicamento se midió mediante un análisis de proliferación celular (Figuras 1- 4). El análisis CellTiter-Glo® Luminescent Cell Viability es un método de análisis homogéneo, disponible en el comercio (Promega Corp., Madison, WI), basado en la expresión recombinante de luciferasa *Coleoptera* (US 5583024; US 5674713; US 5700670). Este análisis de proliferación celular determina el número de células viables en el cultivo, basándose en la cuantificación del ATP presente, un indicador de células metabólicamente activas (Crouch et al (1993) J. Immunol. Meth. 160:81-88; US 6602677). El análisis CellTiter-Glo® se condujo en un formato de 96 pocillos, haciéndolo sensible a cribado automatizado de alto rendimiento (HTS)

(Cree et al (1995) Anticancer Drugs 6:398- 404). El procedimiento de análisis homogéneo involucra agregar el reactivo solo (CellTiter-Glo® Reagent) directamente a células cultivadas en medio suplementado con suero. No se requieren el lavado de las células, el retiro del medio y los múltiples pasos de medición con pipeta. El sistema detecta sólo 15 células/pocillo en un formato de 384 pocillos en 10 minutos luego de agregar el reactivo y mezclar.

El formato homogéneo "agregar-mezclar-medir" resulta en lisis celular y generación de una señal luminescente proporcional a la cantidad de ATP presente. La cantidad de ATP es directamente proporcional al número de células presentes en el cultivo. El análisis CellTiter-Glo® genera una señal luminescente "tipo destello", producida por la reacción de luciferasa, que tiene una semivida generalmente superior a cinco horas, dependiendo del tipo de célula y el medio empleado. Células viables se reflejan en unidades de luminiscencia relativa (RLU). El sustrato, luciferina de escarabajo, se descarboxila oxidativamente por luciferasa de luciérnaga recombinante con conversión concomitante de ATP en AMP y generación de fotones. La semivida extendida elimina la necesidad de usar inyectores de reactivo y brinda flexibilidad para el procesamiento de modo continuo o en lotes de múltiples placas. Este análisis de proliferación celular puede utilizarse con varios formatos multi-pocillo, *por ejemplo*, formato de 96 ó 384 pocillos. Los datos pueden registrarse mediante un luminómetro o dispositivo radiográfico con cámara CCD. La cantidad de luminescencia se presenta como unidades lumínicas relativas (RLU), medidas en el transcurso del tiempo.

$$\text{ATP} + \text{Luciferina} + O_2 \xrightarrow[Mg+2]{\text{Luciferasa}} \text{Oxiluciferina} + \text{AMP} + \text{PPI} + CO_2 + \text{luz}$$

Los efectos antiproliferativos de tres conjugados anticuerpo-medicamento se cuantificaron mediante proliferación celular, análisis de

eliminación celular *in vitro* anterior contra cuatro diferentes líneas celulares de tumor de mama (Figuras 1-4). Figura 1 muestra las mediciones de potencia con concentraciones crecientes de trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 y trastuzumab-SMCC-DM1 luego de un tratamiento de 3 días en células de tumor de mama SK-BR-3 (HER2 3+). Figura 2 muestra las mediciones de potencia con concentraciones crecientes de trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 y trastuzumab-SMCC-DM1 luego de un tratamiento de 3 días en células de tumor de mama BT-474 (HER2 3+). Figura 3 muestra las mediciones de potencia con concentraciones crecientes de trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 y trastuzumab-SMCC-DM1 luego de un tratamiento de 3 días en células de tumor de mama MCF7 (HER2 bajo). Figura 4 muestra las mediciones de potencia con concentraciones crecientes de trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 y trastuzumab-SMCC-DM1 luego de un tratamiento de 3 días en células de tumor de mama MDA-MB-468 (HER2 negativo).

Se establecieron valores IC_{50} para SK-BR-3 y BT-474, los cuales son conocidos por sobreexpresar la proteína de receptor HER2. Un lote de conjugado trastuzumab-SPP-DM1 con 2.8 DM1 por trastuzumab (medicamento/Ab) arrojó una IC_{50} media de 14.4 μg/ml con un rango de 9.1 a 22.3 μg/ml para 6 experimentos contra células SK-BR-3 y una IC_{50} media de 51.7 μg/ml con un rango de 28.7 a 63.1 μg/ml para 4 experimentos contra células BT-474 cells. Un lote de conjugado trastuzumab-SMCC-DM1 con 2.7 DM1 por trastuzumab (medicamento/Ab) arrojó una IC_{50} media de 15.2 μg/ml con un rango de 12.6 a 18.8 μg/ml para 4 experimentos contra células SK-BR-3 y una IC_{50} media de 94.9 μg/ml con un rango de 75.2 a 114.6 μg/ml para 2 experimentos contra células BT-474. Los conjugados fueron inactivos contra células MCF7 y MDA-MB-468, que no sobreexpresan HER2.

AntiCD19-SMCC-DM1 mostró una potente eliminación celular *in vitro* con células Raji (IC_{50} = <0.25 μg/ml) donde el anticuerpo puro ADC de control, trastuzumab-SMCC-DM1 no mostró efecto. AntiCD79a-

SMCC-DM1 y antiCD79b-SMCC-DM1 mostraron una potente eliminación celular *in vitro* con células Ramos (IC_{50} = <0.25 μg/ml) donde el anticuerpo puro y el ADC de control, trastuzumab-SMCC-DM1 no mostraron efecto.

Figura 17 muestra un análisis de proliferación celular, *in vitro*, (Ejemplo 5) con células HT1080EphB2 (C8) tratadas con conjugados anticuerpo antiEphB2R-medicamento: 2H9-SPP-DM1 (IC_{50} 80 ng/ml), y 2H9-SMCC-DM1 (IC_{50} 50 ng/ml).

DEPURACIÓN SÉRICA IN VIVO Y ESTABILIDAD EN RATONES

La depuración sérica y la estabilidad de los ADC se investigaron en ratones lampiños, no sometidos previamente a experimentación (sin tumores recibidos por injertos exógenos). Figura 5 muestra la depuración sérica, en ratones beige lampiños sin tumores, de trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, midiendo la concentración sérica de conjugado y anticuerpo total en seis puntos de tiempo durante 7 días. Una diferencia en la cantidad de anticuerpo total y ADC indica segmentación del enlazador y separación del anticuerpo de su fracción DM1. Tal como se ilustra por la mayor concentración sérica de anticuerpo conjugado en siete días, ADC enlazado a SMCC permaneción intacto *in vivo* mucho más tiempo que los conjugados enlazados a SPP.

Figura 6 muestra la estabilidad en el tiempo, en ratones lampiños sin tumores, de los conjugados: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, y trastuzumab-SMCC-DM1, mediendo la concentración sérica en seis puntos de tiempo durante 7 días. Los ADC con el enlazador SMCC fueron más estables *in vivo* que los conjugados enlazados por SPP o SPDP, aunque el trastuzumab-SMCC-DM1 tuvo aproximadamente la misma estabilidad que el conjugado disulfuro más entorpecido, trastuzumab-SPP-DM4. Los experimentos mostrados en Figuras 5 y 6 se realizaron en ratones lampiños sin tumores. No obstante, los conjugados enlazados a SMCC evidenciaron la misma estabilidad incrementada, comparados con conjugados enlazados a SPP en ratones lampiños con tumores. Tal como se muestra en Figura 7,

aproximadamente 72% del trastuzumab-SMCC-DM1 inicial permaneció como conjugado 7 días después del tratamiento, mientras que sólo aproximadamente 10% del trastuzumab-SPP-DM1 inicial permaneció como conjugado 7 días después del tratamiento, ilustrando la mayor estabilidad de conjugados trastuzumab-SMCC-DM1 no enzimáticamente segmentables en ratones lampiños con tumores.

DEPURACIÓN SÉRICA IN VIVO Y ESTABILIDAD EN RATAS

Figuras 8 y 9 muestran la estabilidad comparativa y los perfiles de depuración de un ADC de enlazador disulfuro (trastuzumab-SPP-DM1 y un ADC de enlazador no disulfuro (trastuzumab-SMCC-DM1) en ratas. Los parámetros del estudio incluyen:

Parámetro	Trastuzumab-SPP-DM1	Trastuzumab-SMCC-DM1
Vd (ml/kg)	41	41
Clearance (ml/día/kg)	52	15
T ½ alpha	0.09	0.15
T ½ beta	0.7	0.85
T ½ gamma (días)	2.6	5.5

El ADC de enlazador no disulfuro, trastuzumab-SMCC-DM1 (Figura 9), mostró una mejor estabilidad en suero de rata que el ADC de enlazador disulfuro, trastuzumab-SPP-DM1 (Figura 8).

EFICACIA IN VIVO

La eficacia de los conjugados anticuerpo-medicamento de la invención puede medirse *in vivo* implantando aloinjertos o xenoinjertos de células cancerosas en roedores y tratando los tumores con ADC. Hay que esperar resultados variables dependiendo de la línea celular, la especificidad de unión del anticuerpo del ADC a receptores presentes en las células cancerosas, el régimen posológico, y otros factores. La eficacia *in vivo* del ADC anti-HER2 se midió mediante un modelo en ratón de explante

transgénico con alta expresión de HER2. Un aloinjerto se propagó del ratón transgénico FoS mmtv, que no responde, o responde a medias a la terpia con HERCEPTIN. Los sujetos se trataron una vez con ADC y se monitorearon durante 3-6 semanas para medir el tiempo a la duplicación del tuumor, la eliminación celular logarítmica (***"log cell kill"***), y el encogimiento del tumor. Se condujeron un seguimiento de la respuesta a la dosis experimentos multi-dosis.

Tumores se originan fácilmente en ratones transgénicos que expresan una forma activada mutacionalmente de *neu*, el holólogo de rata de HER2, pero el HER2 que se se sobreexpresa en neoplasias malignas de mama no es mutado y la formación del tumor es mucho menos robusta en ratones transgénicos, que sobreexpresan HER2 no mutado (Webster et al (1994) Semin. Cancer Biol. 5:69-76).

Para estimular la formación del tumor con HER2 no mutado, se produjeron ratones transgénicos usando un plásmido de ADNc de HER2, en el cual una ATG corriente arriba se eliminó con el fin de prevenir la iniciación de la traducción en tales codones ATG corriente arriba, lo cual reduciría de lo contrario la frecuencia de iniciación de la traducción del codón de iniciación auténtica corriente abajo de HER2 (por ejemplo, véase Child et al (1999) J. Biol. Chem. 274: 24335- 24341). Adicionalmente, se agregó un intrón quimérico al extremo 5', lo cual debería intensificar también el nivel de expresión como se informó anteriormente (Neuberger and Williams (1988) Nucleic Acids Res. 16: 6713; Buchman and Berg (1988) Mol. Cell. Biol. 8: 4395; Brinster et al (1988) Proc. Natl. Acad. Sci. USA 85:836). El intrón quimérico se derivó de un vector Promega, vector de expresión mamífero pCI-neo (bp 890-1022). El extremo 3' de ADNc está flanqueado por exones 4 y 5 de la somatotropina humana, y secuencias de poliadenilación. Más aún, se usaron ratones FVB porque esta cepa es más susceptible al desarrollo de tumores. El promotor de MMTV-LTR se utilizó para garantizar la expresión HER2 específica de tejido en la glándula mamaria. A los animales se les administró la dieta AIN 76A con el fin de

aumentar la susceptibilidad a la formación de tumores (Rao et al (1997) Breast Cancer Res. and Treatment 45:149-158).

Figuras 10-13 muestran que los ADC poseen fuerte actividad anti-tumoral en el aloinjerto de un tumor HER2 positivo (Fo5), que surgió originalmente en un ratón transgénico MMTV-HER2. El anticuerpo solo (*por ejemplo*, trastuzumab) no tiene actividad anti-tumoral significativa en este modelo (Erickson et al US 6632979). Como se ilustra en Figuras 10 y 11, el crecimiento de los tumores se retardó por el tratamiento con ADC, comparado con el nivel de crecimiento del control (vehículo). El crecimiento del tumor se desaceleró en su mayor parte por el tratamiento con conjugados trastuzumab-SMCC-DM1 trastuzumab-SIAB-DM1. Tal como se muestra en Figuras 10, 12 y 13, el conjugado trastuzumab-SMCC-DM1 desaceleró el crecimiento del tumor más que los conjugados con enlazadores SPP, *es decir*, mostró más potencia, ya sea medido como tiempo de duplicación de los tumores en ratones lampiños o como Eliminación Celular Logarítmica correspondiente a las mediciones del tiempo de duplicación mostradas (Figura 13).

La eficacia *in vivo* de ADC anti-CD22 se midió con un modelo de xenoinjerto de tumor de ratón. Grupos de ocho ratones SCID, con 20 millones de células tumorales de xenoinjerto Bjab-luc (células Bjab que expresan luciferasa) por ratón, se dosificaron una vez el día 1 (excepto donde se anotó) con un conjugado anticuerpo anti-CD22-medicamento, o un anticuerpo puro (Ejemplo 8).

ADC ó Ab	µg DM1/m2	Ab mg / kg ratón	Carga media de medicamento (p)	MTD (días)
Tr-SMCC-DM1	200	4.2	3.2	3
7A2-SMCC-DM1	200	3.8	3.6	6
5E8-SMCC-DM1	200	3.8	3.6	10
RFB4-SMCC-DM1	200	3.2	4.25	18
RFB4-SMCC-DM1 (dosificado 3X los días 1, 7, 14)	405	10	2.75	55

7A2	-	4	-	3
5E8	-	4	-	3
RFB4	-	4	-	3

Se midió el tiempo requerido para doblar el tamaño del tumor (MTD, tiempo medio de duplicación del tumor). Los tres anticuerpos anti-CD22 puros no mostraron esencialmente ninguna eficacia en ralación con un ADC de unión no específica (trastuzumab-SMCC-DM1). Todos los conjugados correspondientes mostraron el efecto de retardar significativamente el crecimiento del tumor. El efecto de la dosificación múltiple se estableció con RFB4-SMCC-DM1, donde el MTD para ratones dosificados por una sola vez fue de 18 días, mientras que el MTD para ratones medicados tres veces, los días 1, 7, y 14, fue de 55 días. La remisión completa del tumor ocurrió en todos los 8 ratones en el grupo que recibió una dosis triple. Otros conjugados antiCD22-SMCC-DM1, con anticuerpos 12F7, 9A8, 8C9, 8G10, 3F11, 10D2, 6C9, 14D1, y HH10, mostraron encogimiento del volumen del tumor inicial o retardo del crecimiento del tumor en relación con el control (trastuzumab-SMCC-DM1), después de 7 días luego de una dosis única (400 μg DM1/m2) en ratones SCID con 20 millones de células tumorales de xenoinjerto Bjab-luc por ratón. Conjugados antiCD22, RFB4-SMCC-DM1, 5E8-SMCC-DM1, y 7A2-SMCC-DM1 fueron igualmente efectivos en el retardo del crecimiento del tumor en relación con el control (trastuzumab-SMCC-DM1), después de 11 días luego de una dosis única (200 μg DM1/m2) en ratones SCID con 5 millones de células tumorales de xenoinjerto RA1 Ramos por ratón.

El conjugado RFB4-SMCC-DM1 se estudió con tres diferentes cargas de medicamento en grupos de diez ratones SCID con xenoinjertos Bjab-luc (Ejemplo 8). Los conjugados con carga de medicamento baja (1.95) y media (3.7) mostraron cada uno el efecto de retardar significativamente el crecimiento del tumor, con un MTD de alrededor de 15 días. El conjugado de carga elevada (6.75) no mostró un efecto

significativamente diferente del conjugado de control GP120-SMCC-DM1, o anticuerpo REB4 puro.

anti-CD22 ADC ó Ab	µg DM1/m2	Ab mg / kg ratón	Carga media de medicamento (p)	MTD (días)
RFB4	-	10	-	3
RFB4-SMCC-DM1 (carga baja)	144	5	1.95	15
RFB4-SMCC-DM1 (carga media)	273	5	3.7	15
RFB4-SMCC-DM1 (carga alta)	497	5	6.75	3
GP120-SMCC-DM1 (carga alta)	449	5	6.1	3

Conjugados antiCD19-SMCC-DM1 y antiCD22-SMCC-DM1 no mostraron actividad *in vivo* en un modelo de xenoinjerto de tumor de ratón con células Raji. Otros conjugados antiCD19 y antiCD22 pueden tener actividad *in vivo* contra otros modelos de tumor de células cancerosas.

La eficacia *in vivo* de ADC anti-CD79a (alfa) y anti-CD79b (beta) se midió con un modelo de xenoinjerto de tumor de ratón. Grupos de ocho ratones SCID con 20 millones de células tumorales de xenoinjerto Bjab-luc por ratón se dosificaron el día 1 con muestras en la tabla y el siguiente Ejemplo 8.

ADC, Ab, o control	µg DM1/m2	Ab mg / kg ratón	Carga media de medicamento (p)	MTD (días)
PBS (control de tampón)	-	-	-	3.5
antiGP120	-	3.2	-	3.5
SN8 antiCD79b	-	3.1	-	4
17A7 antiCD79b	-	3.1	-	4
8H9 antiCD79a	-	4.0	-	3
antiGP120-SMCC-DM1	200	3.2	4.2	3.5
SN8 antiCD79b-SMCC-DM1	200	3.1	4.4	>7

17A7 antiCD79b-SMCC-DM1	200	3.1	4.4	>7
8H9 antiCD79a-SMCC-DM1	200	4.0	3.4	>7

Todos los conjugados SN8 antiCD79b-SMCC-DM1, 17A7 antiCD79b-SMCC-DM1, y 8H9 antiCD79a-SMCC-DM1 mostraron encogimiento del volumen del tumor inicial (media 160 mm3) después de 7 días. En los grupos de 8 ratones, el conjugado SN8 antiCD79b-SMCC-DM1 produjo la remisión parcial (PR) en 4 animales y remisión completa (CR) en 2 animales. El conjugado 17A7 antiCD79b-SMCC-DM1 produjo CR en 1 animal. El conjugado 8H9 antiCD79a-SMCC-DM1 produjo PR en 2 animales y CR en 1 animal. Otros conjugados antiCD79b-SMCC-DM1, con anticuerpos 2F2, 5C3, 7H7, 8D11, 15E4, y 16C11, mostraron retardo del crecimiento del tumor o encogimiento del volumen del tumor inicial, en relación con el control (trastuzumab-SMCC-DM1), después de 8 días luego de una dosis única (192 µg DM1/m2) en ratones CB 17 ICR SCID con 20 millones de células tumorales de xenoinjerto Bjab-luc por ratón.

Se midió el efecto de respuesta a la dosis sobre ratones a los que se había administrado antiCD79b-SMCC-DM1. Grupos de ocho ratones SCID con 20 millones de células tumorales de xenoinjerto Bjab-luc por ratón se dosificaron el día 1 con muestras en la siguiente tabla (Ejemplo 8). El antiCD79b-SMCC-DM1 se administró a niveles de 0.5, 2.0, y 3.64 mg Ab/kg ratón.

ADC, Ab, o control	µg DM1/m2	Ab mg / kg ratón	MTD (días)
Control vehículo (PBS)	-		4
antiCD79b-SMCC-DM1	32	0.5	10
antiCD79b-SMCC-DM1	130	2.0	35
antiCD79b-SMCC-DM1	236	3.64	>70

La eficacia *in vivo* de ADC antiTENB2 se midió con un modelo de xenoinjerto de tumor de ratón. TENB2 es un antígeno tumoral que ha demostrado que se expresa casi exclusivamente en la próstata humana y se sobreexpresa en tumores de próstata humana (Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84). PC3-TVA-919cv1:5 es una línea celular de cáncer de próstata humana que expresa un nivel alto de TENB2.

Ratones lampiños atímicos se inyectaron subcutáneamente con 5 millones de células expresoras medias o expresoras altas PC3-TVA-919 en un volumen de 0.2 ml por ratón. Las células se suspendieron en HBSS. Cuando el tamaño de tumor medio alcanzó 100-200 mm^3, los ratones se agruparon aleatoriamente en 8 grupos de 8-10 ratones cada uno y se les dio un solo tratamiento IV de las siguientes muestras (Ejemplo 8).

ADC o control	µg DM1/m2	Ab mg / kg ratón	MTD expresora media (días)	MTD expresora alta (días)
PBS (control vehículo)	-	-	30	22
anti-ragweed-SPP-DM1 murino (control negativo)	373	6.68	18	22
anti-ragweed-SMCC-DM1 murino (control negativo)	373	9.34	14	18
antiTENB2:3146-SPP-DM1 murino	373	7.34	58	35
antiTENB2:3146-SMCC-DM1 murino	373	10.6	44	19
antiTENB2-SPP-DM1 quimérico	373	8.7	57	43
antiTENB2-SMCC-DM1 quimérico	373	7.63	43	22

Conjugados anti-TENB2-DM1 murino mostraron eficacia anti-tumoral contra tumores PC3-TENB2, en relación con el control negativo y el control vehículo. El conjugado 10H1 antiNaPi3b-SMCC-DM1 murino no mostró eficacia anti-tumoral contra tumores PC3-NaPi3b, en relación con control negativo y control vehículo. Otras variantes de anticuerpo de

conjugados antiNaPi3b pueden tener actividad *in vivo* contra tumores PC3-NaPi3b, u otras líneas celulares cancerosas.

TOXICIDAD A ROEDORES

Los conjugados anticuerpo-medicamento y un control sin ADC, "vehículo", se evaluaron en un modelo de rata de toxicidad aguda. Se investigó la toxicidad del ADC por tratamiento de ratas Sprague-Dawley hembras con el ADC e inspección posterior y análisis de los efectos sobre varios órganos. Basándose en observaciones aproximadas (pesos corporales), parámetros de patología clínica (química del suero y hematología) e histopatología, puede observarse la toxicidad de ADC, caracterizarse y medirse. Se descubrió que, con niveles de dosis equivalentes, trastuzumab-SMCC-DM1 se asoció con toxicidad menos aguda que trastuzumab-SPP-DM1.

Se condujo un estudio de toxicidad aguda de 5 días en ratas hembra adolescentes (100-125 gms) con una inyección única de trastuzumab-SMCC-DM1 (dos dosis: 1860 y 3260 μg DM1/m^2), un ADC de disulfuro de comparación, trastuzumab-SPP-DM1 (dos dosis: 1860 y 3260 μg DM1/m^2), maitansina DM1 libre (tiol) y un vehículo de control (día 0). Todos los días se tomó el peso corporal. Se condujeron química clínica, enzimas séricas y análisis de hematología los días 3 y 5; concluyendo con necropsia completa con evaluación histopatológica. Las señales de toxicidad incluyeron la observación clínica de la pérdida de peso.

Se considera que la pérdida de peso, o cambio de peso relativo a animales dosificados únicamente con vehículo en animales luego de la dosificación con ADC, es un indicador general y aproximado de toxicidad sistémica o localizada. Figura 14 muestra los cambios en peso corporal (gramos) durante 5 días. Ratas que recibieron el ADC de disulfuro, trastuzumab-SPP-DM1 mostraron un marcada toxicidad dependiente de la dosis, indicada por la letalidad con una dosis más alta y un descenso en el peso corporal con la dosis más baja. En contraste, ratas que recibieron trastuzumab-SMCC-DM1 ganaron peso, mostrando las ratas dosificadas

con la dosis menor ningún descenso en la tasa de ganancia de peso, relativa a las ratas dosificadas con vehículo placebo. Las ratas dosificadas con el nivel más alto de trastuzumab-SMCC-DM1 también ganaron peso, comparable con la citotoxina DM1 libre.

Se midió la hepatotoxicidad por enzimas hepáticas elevadas, números incrementados de figuras mitóticas y apoptóticas y necrosis de hepatocitos. Se observó toxicidad hematolinfoide por agotamiento de leucocitos, principalmente granulocitos (neutrófilos), y/o plaquetas, e implicación de órganos linfoides, *es decir*, atrofia o actividad apoptótica. Se observó también toxicidad por lesiones del tracto gastrointestinal tales como números aumentados de figuras mitóticas y apoptóticas y enterocolitis degenerativa.

Enzimas indicadoras de lesión hepática, que se estudiaron, incluyen:

AST (aspartato aminotransferasa)

-Localización: citoplásmica; hígado, corazón, músculo esquelético, riñón

-Relación hígado:plasma de 7000:1

-T1/2: 17 horas

ALT (alanina aminotransferasa)

-Localización: citoplásmica; hígado, riñón, corazón, músculo esquelético

-Relación hígado: plasma de 3000:1

-T1/2: 42 horas; variación diurna

GGT (g-glutamil transferasa)

-Localización: membrana plasmática de células con alta capacidad de secreción o absorción: hígado, riñón, intestino

-Predictor deficiente de lesión hepática; comúnmente elevada en trastornos de los ductos biliares

Ninguna de las tres enzimas medidas anteriormente son hepato-específicas. Se descubrió que los ADC de la presente invención ocasionaron una elevación moderada, transitoria, de enzimas hepáticas ALT

y AST, con reticulocitopenia transitoria (Figura 15). No se observó un efecto significativo sobre los granulocitos de la sangre periférica o plaquetas (Figura 16).

Figuras 15 y 16 muestran que las ratas expuestas a 22.3 mg/kg trastuzumab-SPP-DM1, (Grupo 2) exhibieron la toxicidad clínica más seria en este estudio de toxicidad aguda de cinco días. Estos animales mostraron la pérdida de peso corporal más pronunciada, elevaciones de pruebas de función hepática, leuco- y trombocitopenia y evidencia morfológica de toxicidad contra tejidos hematolinfoides. La magnitud de la toxicidad fue similar, comparada con la encontrada en estudios previos usando una dosis de 25mg/kg. En contraste, los animales en los Grupos 3 y 4 que recibieron trastuzumab-SMCC-DM1 a 10 y 25 mg/kg, respectivamente, fueron difíciles de distinguir de los animales tratados con vehículo, basándose en datos de patología clínica y peso corporal. Morfológicamente, estos animales mostraron un número moderadamente aumentado de figuras mitóticas en el hígado; no obstante, los tejidos periféricos linfoides y hematopoyéticos estuieron dentro de los límites normales.

Los animales del Grupo 5, 50 mg/kg trastuzumab-SMCC-DM1, mostraron evidencia de toxicidad. Sin embargo, con la excepción de una prueba de función hepática (ALT), la gravedad de la toxicidad fue menor en animales que recibieron 50% de la misma dosis de medicamento que trastuzumab-SPP-DM1 (Grupo 2). Con casi la misma dosis, trastuzumab-SMCC-DM1 (Group 4, 22.3 mg/kg) mostró alrededor del 25% del nivel AST que trastuzumab-SPP-DM1 (Grupo 2, 25 mg/kg). El día 5 de este estudio, los animales del Grupo 5 mostraron aumentos en los pesos corporales (luego de una pérdida transitoria durante los días 3 y 4), una bilirrubina sérica decreciente y un conteo de plaquetas creciente (Figura 15).

Los animales expuestos a DM1 maitansinoide libre (Grupo 6) muestran el mismo patrón de toxicidad que los animales tratados con conjugados de trastuzumab. Esta dosis de DM1 libre corresponde a la cantidad de medicamento administrado como una dosis de 10 mg/kg

trastuzumab-SPP-DM1 en estudios previos. La gravedad de la toxicidad en los animales del Grupo 6 fue menor que la observada en los animales de Grupo 2, pero mayor que la observada previamente en los animales tratados con 10 mg/kg trastuzumab-SPP-DM1. La recuperación pareció ser más bien rápido: Las secciones de bazo mostraron números crecientes de elementos hematopoyéticos inmaduros en animales tratados con maitansina libre; asímismo, LFTs y parámetros de hematología clínica mostraron una tendencia distinta hacia la normalización en animales de Grupo 6 el día 5.

TOXICIDAD/SEGURIDAD EN MONO CYNOMOLGUS

Puede evalaurse la toxicidad y seguridad de ADC administrados a monos Cynomolgus. Se condujo en monos Cynomolgus un estudio de toxicidad/seguridad del conjugado anticuerpo-medicamento, trastuzumab-SMCC-DM1. Tres grupos de monos se estudiaron para evaluar la toxicidad de trastuzumab-SMCC-DM1, administrado por inyección intravenosa, en dosis cada vez mayores, relativa al control (vehículo). Grupo 1 (4 sujetos) recibieron únicamente vehículo (PBS, pH 6.5, *es decir*, formulación sin ADC) el día 1 y el día 22, seguida por necropsia el día 36. Grupo 2 (4 sujetos) recibieron trastuzumab-SMCC-DM1 4900 μg/m2 el día 1 y el día 22. Grupo 3 (4 sujetos) recibieron trastuzumab-SMCC-DM1 7200 μg/m2 el día 22.

Se infirió la hepatotoxicidad mediante medición de las enzimas hepáticas elevadas del estudio de Toxicidad en Roedores. Monos Cynomolgus se dosificaron con vehículo (Grupo 1) y trastuzumab-SMCC-DM1 (Grupo 2: 4900 $\mu g/m^2$; Grupo 3: 7200 $\mu g/m^2$. AST de enzima hepática, conteos de plaquetas, leucocitos, neutrófilo absoluto, glóbulos rojos, reticulocitos, y una comparación de 2 regímenes de dosis IV se midieron con respecto a trastuzumab-SMCC-DM1 en monos Cynomolgus (Ejemplo 10).

ADMINISTRACIÓN DE FORMULACIONES FARMACÉUTICAS DE CONJUGADO ANTICUERPO-MEDICAMENTO

Conjugados terapéuticos anticuerpo-medicamento (ADC) pueden administrarse por cualquier ruta apropiada a la enfermedad a tratarse. El ADC se administrará típicamente por vía parenteral, *es decir*, infusión, inyección subcutánea, intramuscular, intravenous, intradermal, intratecal, inyección intravenosa rápida, inyección intratumoral o epidural (Shire et al (2004) J. Pharm. Sciences 93(6):1390-1402). Formulaciones farmacéuticas de conjugados terapéuticos anticuerpo-medicamento (ADC) se preparan típicamente para administración parenteral con un vehículo parenteral farmacéuticamente aceptable y en una forma inyectable de dosificación unitaria. Un conjugado anticuerpo-medicamento (ADC), que tiene el grado deseado de pureza se mezcla opcionalmente con diluyentes, portadores, excipientes o estabilizantes farmacéuticamente aceptables, en la forma de una formulación liofilizada o una solución acuosa (Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.).

Vehículos, diluyentes, portadores, excipientes y estabilizadores parenterales aceptables son atóxicos a los destinatarios en las dosis y concentraciones empleadas, e incluyen tampones tales como fosfato, citrato, y otros ácidos orgánicos; antioxidantes incluidos ácido ascórbico y metionina; preservantes (tales como cloruro de octadecildimetilbencil amonio; cloruro de hexametonio; cloruro de benzalconio, cloruro de benzetonio; fenol, alcohol butílico o bencílico; alquil parabenos tales como metil o propil parabeno; catecol; resorcinol; ciclohexanol; 3-pentanol; y m-cresol); polipéptidos de bajo peso molecular (menos de alrededor de 10 residuos); proteínas, tales como albúmina sérica, gelatina, o inmunoglobulinas; polímeros hidrófilos tales como polivinilpirrolidona; aminoácidos tales como glicina, glutamina, asparagina, histidina, arginina, o lisina; monosacáridos, disacáridos, y otros carbohidratos incluidos glucosa, mannosa, o dextrinas; agentes quelantes tales como EDTA; azúcares tales como sacarosa, mannitol, trehalosa o sorbitol; iones contrarios formadores de sales tales como sodio; complejos de metal (*por*

ejemplo, complejos Zn-proteína); y/o surfactantes no iónicos tales como TWEEN™, PLURONICS™ o polietilen glicol (PEG). Por ejemplo, formulaciones liofilizadas de anticuerpo anti-ErbB2 se describen en WO 97/04801, expresamente incorporada en esta solicitud por referencia. Una formulación ejemplar de un ADC tal como trastuzumab-SMCC-DM1 contiene alrededor de 100 mg/ml de trehalosa (2-(hidroximetil)-6-[3,4,5-trihidroxi-6-(hidroximetil)tetrahidropiran-2-il]oxi-tetrahidropiran-3,4,5-triol; $C_{12}H_{22}O_{11}$; CAS Number 99-20-7) y alrededor de 0.1% TWEEN™ 20 (polisorbato 20; 2-[2-[3,4-bis(2-hidroxietoxi)tetrahidrofuran-2-il]-2-(2-hidroxietoxi)etoxi]etil éster de ácido dodecanoico; $C_{26}H_{50}O_{10}$; CAS Number 9005-64-5) a aproximadamente pH 6.

Formulaciones farmacéuticas de un conjugado terapéutico anticuerpo-medicamento (ADC) pueden contener ciertas cantidades de fracción de medicamento sin reaccionar (D), intermedio anticuerpo-enlazador (Ab-L), y/o intermedio medicamento-enlazador (D-L), como consecuencia de purificación incompleta y separación de exceso de reactivos, impurezas, y subproductos, en el proceso de elaborar ADC; o hidrólisis tiempo/temperatura o degradación con el almacenamiento del ADC a granel o composición ADC formulada. Por ejemplo, una formulación del ADC, trastuzumab-SMCC-DM1 puede contener una cantidad detectable de medicamento DM1 libre. Alternativa, o adicionalmente, ésta puede contener una cantidad detectable de intermedio medicamento-enlazador DM1-SMCC. Alternativa, o adicionalmente, ésta puede contener una cantidad detectable del anticuerpo, trastuzumab. Una formulación ejemplar de trastuzumab-SMCC-DM1 puede contener hasta 10% equivalente molar de DM1-SMCC. Inesperadamente, se determinó mediante el análisis de proliferación celular *in vitro* (Ejemplo 5), que DM1-SMCC (IC_{50} 0.05 μM) es alrededor de 20 veces menos potente en eliminar células que el medicamento libre DM1 (IC_{50}0.0045 μM) contra células de cáncer de mama SK-BR-3 y BT-474.

Los ingredientes farmacéuticos activos pueden atraparse también en microcápsulas preparadas, por ejemplo, por técnicas coacervación o por

polimerización interfacial, por ejemplo, hidroximetilcelulosi o microcápsulas de gelatina y microcápsulas de poli-(metilmetacilato), respectivamente, en sistemas coloidales de liberación de medicamento (por ejemplo, liposomas, microesferas de albúmina, microemulsiones, nanopartículas y nanocápsulas) o en macroemulsiones. Tales técnicas se divulgan en Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

Pueden prepararse preparaciones de liberación sostenida. Ejemplos apropiados de preparaciones de liberación sostenida incluyen matrices semi-permeables de polímeros hidrófobos sólidos que contienen el ADC, matrices que están en la forma de artículos moldeados, *por ejemplo*, películas, o microcápsulas. Ejemplos de matrices de liberación sostenida incluyen poliésteres, hidrogeles (por ejemplo, poli(2-hidroxietil-metacrilato), o poli(vinilalcohol)), polilácticos (US 3773919), copolímeros de ácido L-glutámico y gamma-etil-L-glutamato, etilen-vinil acetato no degradable, copolímeros degradables ácido láctico-ácido glicólico tales como el LUPRON DEPOT™ (microesferas inyectables compuestas de copolímero ácido láctico-ácido glicólico y acetato de leuprolida), y ácido poli-D-(-)-3-hidroxibutírico.

Las formulaciones a usarse para administración *in vivo* tienen que ser estériles, lo cual se obtiene fácilmente por filtración a través de membranas de filtración estériles. Las formulaciones incluyen aquellas apropiadas para las anteriores vías de administración. Las formulaciones pueden presentarse convenientemente en forma farmacéutica unitaria y pueden prepararse por cualquiera de los métodos conocidos en el arte de la farmacia. Técnicas y formulaciones se encuentran por lo general en *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, PA). Tales métodos incluyen el paso de poner en asociación el ingrediente activo con el portador que constituye uno o más ingredientes accesorios. En general, las formulaciones se preparan poniendo en asociación uniforme e íntima el ingrediente activo con portadores líquidos o portadores sólidos

finamente divididos o ambos y, luego, si es necesario, moldeando el producto.

Suspensiones acuosas contienen los materiales activos (ADC) en mezcla con excipientes apropiados para la fabricación de suspensiones acuosas. Tales excipientes incluyen un agente de suspensión, tal como carboximetilcelulosa sódica, croscarmelosa, povidona, metilcelulosa, hidroxipropilmetilceluosa, alginato de sodio, polivinilpirrolidona, goma tragacanto y goma de acacia, y agentes humectantes o dispersantes tales como fosfátidos de ocurrencia natural (*por ejemplo*, lecitina), un producto de condensación de un óxido de alquileno con un ácido graso (*por ejemplo*, estearato de polioxietileno), un producto de condensación de óxido de etileno con un alcohol alifático de cadena larga (*por ejemplo*, heptadecaetilenoxicetanol), un producto de condensación de óxido de etileno con un éster parcial derivado de un ácido graso y un anhídrido de hexitol (*por ejemplo*, polioxietilen sorbitán monooleato). La suspensión acuosa puede contener asimismo uno o más preservantes como etil o n-propil p-hidroxi-benzoato, uno o más agentes colorantes, uno o más agentes saborizantes y uno o más agentes edulcorante, tales como sacarosa o sacarina.

Las composiciones farmacéuticas de ADC pueden estar en la forma de una preparación inyectable estéril, tal como una suspensión acuosa u oleaginosa inyectable estéril. Esta suspensión puede formularse de acuerdo con el arte conocido usando aquellos agentes dispersantes o humectantes adecuados y agentes de suspensión, que ya han sido mencionados. La preparación inyectable estéril puede ser también una solución inyectable estéril o suspensión en un diluyente o disolvente atóxico parenteralmente aceptable, tal como una solución en 1,3-butano-diol o preparada como un polvo liofilizado. Entre los vehículos y disolventes aceptables, que pueden emplearse se encuentran agua, solución de Ringer y solución de cloruro de sodio isotónico. Adicionalmente, aceites fijados estériles pueden empelarse convencionalmente como medio disolvente o de suspensión. Para este efecto puede emplearse cualquier aceite fijado suave, incluidos mono o

diglicéridos sintéticos. Además, ácidos grasos tales como ácido oleico pueden emplearse igualmente en la preparación de inyectables.

La cantidad de ingrediente activo que puede combinarse con el material portador para producir una forma farmacéutica única variará dependiendo del huésped tratado y el modo particular de administración. Por ejemplo, una solución acuosa prevista para infusión intravenosa puede contener desde alrededor de 3 a 500 μg del ingrediente activo por milímetro de solución con el fin de que pueda ocurrir infusión de un volumen apropiado a una velocidad de alrededor de 30 mL/hora. Puede efectuarse administración subcutánea (inyección intravenosa rápida) con alrededor de 1.5 ml, o menos, del volumen total y una concentracion de alrededor de 100 mg ADC por ml. Para ADC que requieren una administración frecuente y crónica, puede emplearse la ruta subcutánea, tal como una jeringa previamente llenada o tecnología de dispositivo autoinyector.

Como proposición general, la cantidad inicial farmacéuticamente efectiva de ADC administrado por dosis estará en el rango de alrededor de 0.01-100 mg/kg, a saber, alrededor de 0.1 a 20 mg/kg de peso corporal del paciente por día, siendo el rango inicial típico de compuesto 0.3 a 15 mg/kg/día. Por ejemplo, pacientes humanos pueden medicarse inicialmente con alrededor de 1.5 mg ADC por kg de peso corporal del paciente. La dosis puede aumentarse a la dosis máxima tolerada (MTD). La pauta posológica puede ser aproximadamente cada 3 semanas pero, de acuerdo con la enfermedad diagnosticada o respuesta, la pauta puede ser más o menos frecuente. Además las dosis puede ajustarse durante el ciclo de tratamiento para que esté por debajo de la MTD, que puede adminsitrarse con seguridad durante ciclos múltiples, tales como alrededor de 4 ó más.

Formulaciones apropiadas para administración parenteral incluyen soluciones de inyección estéril acuosa y no acuosa, que pueden contener anti-oxidantes, tampones, bacteriostáticos y solutos que vuelven la formulación isotónica con la sangre del destinatario previsto; y suspensiones estériles acuosas y no acuosas, que pueden incluir agentes de suspensión y agentes espesantes.

Aunque la administración oral de terapéuticos proteínicos se ve generalmente desfavorecida debido a escasa biodisponibilidad en razón de una absorción limitada, hidrólisis o desnaturalización en el intestino, las formulaciones de ADC adecuadas para administración oral pueden prepararse como unidades discretas tales como cápsulas, sellos o tabletas, cada una de las cuales contiene una cantidad predeterminada del ADC.

Las formulaciones pueden empacarse en envases de dosis única o dosis múltiple, por ejemplo ampolletas selladas y frasquitos, y pueden almacenarse en estado secado por congelamiento (liofilizado) que requiere únicamente la adición del portador líquido estéril, por ejemplo agua, para inyección inmediatamente antes del uso. Solucioones para inyección extemporánea y suspensiones se preparan de polvos estériles, gránulos y tabletas de la clase descrita anteriormente. Formulaciones ejemplares de dosificación única contienen una dosis diaria o subdosis diaria unitaria, o una fracción apropiada de la misma, del ingrediente activo.

La invención provee además composiciones veterinarias que comprenden por lo menos un ingrediente activo, como se define anteriormente, junto con un portador veterinario para el mismo. Portadores veterinarios son materiales útiles para el propósito de administrar la composición y pueden ser materiales sólidos, líquidos o gaseosos que, de lo contrario, son inertes o aceptables en el arte veterinario y son compatibles con el ingrediente activo. Estas composiciones veterinarias pueden administrarse por vía parenteral, oral o por cualquier otra ruta deseada.

TRATAMIENTOS CON CONJUGADO ANTICUERPO-MEDICAMENTO

Se contempla que los conjugados anticuerpo-medicamento (ADC) de la presente invención pueden usarse para tratar varias enfermedades y trastornos, tales como cáncer y afecciones autoinmunitarias. Afecciones ejemplares o trastornos incluyen tumores benignos o malignos; leucemia y neoplasias malignas linfoides; otros trastornos tales como trastornos neuronales, gliales, astrocíticos, hipotalámicos, glandulares, macrofágicos,

epiteliales, estromales, blastocélico, inflamatorios, angiogénicos e inmunológicos. Tipos de cáncer susceptibles a tratamiento con ADC incluyen aquellos que se caracterizan por la sobreexpresión de ciertos antígenos asociados con tumores o receptores de superficie celular, *por ejemplo*, HER2

Los compuestos ADC, que se identifican en los modelos con animales y análisis basados en células, pueden analizarse adicionalmente en primates más altos portadores de tumores y estudios clínicos humanos. Pueden diseñarse estudios clínicos humanos similares a los estudios clínicos que prueban la eficacia del anticuerpo monoclonal anti-HER2 HERCEPTIN en pacientes con HER2 que sobreexpresa neoplasias malignas de mama, metastásicas, los cuales habían recibido una extensiva terapia previa anti-cáncer, tal como se divulga por Baselga et al. (1996) J. Clin. Oncol. 14:737-744. El estudio clínico puede diseñarse para evaluar la eficacia de un ADC en combinación con regímenes terapéuticos conocidos, tales como radiación y/o quimioterapia que involucra agentes quimioterapéuticos y/o citotóxicos conocidos (Pegram et al (1999) Oncogene 18:2241-2251).

Generalmente, la enfermedad o trastorno a tratarse es cáncer. Ejemplos de cáncer a tratarse en esta solicitud incluyen, pero no se limitan a, carcinoma, linfoma, blastoma, sarcoma, y leucemia o neoplasias malignas linfoides. Ejemplos más particulares de tales neoplasias malignas incluyen carcinoma epidermoide (*por ejemplo*, carcinoma epidermoide epitelial), cáncer de pulmón, incluidos cáncer de pulmón microcítico, cáncer de pulmón amicrocítico ("NSCLC"), adenocarcinoma del pulmón y carcinoma epidermoide del pulmón, cáncer del peritoneo, cáncer hepatocelular, cáncer gástrico o del estómago incluido cáncer gastrointestinal, tumor estromal gastrointestinal (GIST), cáncer pancreático, glioblastoma, cáncer cervical, cáncer de ovarios, cáncer del hígado, cáncer de la vejiga, hepatoma, cáncer de mama, cáncer de colon, cáncer rectal, cáncer colorrectal, cáncer del endometrio o carcinoma uterino, carcinoma de glandula salival, cáncer de riñón o renal, cáncer de próstata, cáncer de la vulva, cáncer de la tiroides,

carcinoma hepático, carcinoma anal, carcinoma del pene, así como cáncer de cabeza y cuello.

El cáncer a tratarse en esta solicitud puede ser uno caracterizado por activación excesiva de un receptor ErbB, *epor ejemplo*, HER2. Tal activación excesiva puede ser atribuíble a la sobreexpresión o producción aumentada del receptor ErbB o un ligando ErbB. En una incorporación, se realizará un análisis diagnóstico o prognóstico para determinar si el cáncer del paciente se caracteriza por activación excesiva de un receptor ErbB. Por ejemplo, puede determinarse amplificación génica de ErbB y/o sobreexpresión de un receptor ErbB en el cáncer. Varios análisis para determinar tal amplificación/sobreexpresión están disponibles en el arte e incluyen análisis IHC, FISH y análisis de antígeno circulante descritos más arriba. Alternativa, o adicionalmente, niveles de un ligando ErbB, tal como TGF-alfa, en o asociados con el tumor, pueden determinarse de acuerdo con procedimientos conocidos. Tales análisis pueden detectar proteína y/o ácido nucleico que la codifica en la muestra a probarse. En una incorporación, niveles de ligando ErbB en el tumor pueden determinarse usando inmunohistoquímica (IHC); véase, por ejemplo, Scher et al. (1995) Clin. Cancer Research 1:545-550. Alternativa, o adicionalmente, se pueden evaluar niveles de de ácido nucleico que codifica ligando ErbBen la muestra a probarse; *por ejemplo*, mediante FISH, southern blotting, o técnicas PCR. En una incorporación, la sobreexpresión de ErbB2 puede analizarse por IHC, *por ejeinplo*, usando el HERCEPTEST (Dako). Secciones de tejido incrustadas en parafina de una biopsia de tumor pueden someterse al análisis IHC y asignárseles criterios de intensidad de tinción de proteína ErbB2 en la siguiente forma: Calificación 0, no se observó tinción o tinción de membrana en menos del 10% de las células tumorales; Calificación 1+, se detectó una tinción de membrana débil/apenas perceptible en más del 10% de las células tumorales, las células están teñidas únicamente en parte de su membrana; Calificación 2+, se observó una tinción de membrana completa, débil a moderada en más de 10% de las células tumorales; Calificación 3+, se observó una tinción de membrana

completa, moderada a fuerte, en más del 10% de las células tumorales. Aquellos tumores con calificaciones de 0 ó 1+ para la evaluación de la sobreexpresión de ErbB2 pueden caracterizarse como no tumores que no sobreexpresan ErbB2, mientras que aquellos tumores con calificaciones 2+ ó 3+ pueden caracterizarse como tumores que sobreexpresan ErbB2.

Alternativa, o adicionalmente, análisis FISH tales como el INFORM™ (Ventana Co., Ariz.) o PATHVISION™ (Vysis, 111.) pueden llevarse a cabo en tejido tumoral fijado en formalina, incrustado en parafina, para determinar la magnitud (si la hubiere) de la sobreexpresión de ErbB2 en el tumor.

Más aún, la sobreexpresión o amplificación del receptor ErbB o el ligando ErbB pueden evaluarse usando un análisis diagnóstico *in vivo*, *por ejemplo*, administrando una molécula (tal como un anticuerpo), que une la molécula a detectarse y se marca con un marcador detectable (*por ejemplo*, *un isótopo* radioactivo) escaneando externamente al paciente para localizar el marcador.

Para la prevención o tratamiento de enfermedad, la posología apropiada de un ADC dependerá del tipo de enfermedad a tratarse, como ya se definió, la gravedad y ciclo de la enfermedad, si la molécula se administra con fines preventivos o terapéutico, terapia previa, la historia clínica del paciente y respuesta al anticuerpo, y la discreción del médico a cargo del paciente. La molécula se administra convenientemente al paciente de una vez o durante una serie de tratamientos. Dependiendo del tipo y gravedad de la enfermedad, alrededor de 1 µg/kg a 15 mg/kg (*por ejemplo*, 0.1-20 mg/kg) de la molécula es una posología candidata inicial para administración al paciente, ya sea, por ejemplo, por una o más administraciones separadas, o por infusión continua. Una posología diaria típica podría variar desde alrededor de 1 µg/kg a 100 mg/kg o más, dependiendo de los factores ya mencionados. Una dosificación ejemplar de ADC a administrarse a un paciente está en el rango de alrededor de 0.1 a alrededor de 10 mg/kg de peso del paciente.

Para administraciones repetidas durante varios días o más, dependiendo de la enfermedad, el tratamiento se sostiene hasta cuando ocurra una supresión deseada de la enfermedad. Un régimen posológico ejemplar comprende administrar una dosis de carga inicial de alrededor de 4 mg/kg, seguida por una dosis de mantenimiento semanal de alrededor de 2 mg/kg del anticuerpo anti-ErbB2. Otros regímenes posológicos pueden ser útiles. El avance de esta terapia se monitorea fácilmente por técnicas y análisis convencionales.

TERAPIA DE COMBINACIÓN

Un conjugado anticuerpo-medicamento (ADC) puede combinarse en una formulación de combinación farmacéutica, o régimen de dosificación como terapia de combinación, con un segundo compuesto que posee propiedades anti-neoplásicas. El segundo compuesto de la formulación de combinación farmacéutica o régimen de dosificación tiene preferiblemente actividades complementarias al ADC de la combinación tales que so se afecta negativamente entre sí.

El segundo compuesto puede ser un agente quimioterapéutico, agente citotóxico, citocina, agente inhibidor de crecimiento, agente anti-hormonal, inhibidor de aromatasa, inhibidor de proteína cinasa, inhibidor de cinasa lipídica, anti-andrógeno, oligonucleótido complementario, ribozima, vacuna de terapia génica, agente anti-angiogénico y/o cardioprotector. Tales moléculas se encuentran convenientemente presentes en combinación en cantidades que son efectivas para el propósito previsto. Una composición farmacéutica que contiene un ADC puede tener igualmente una cantidad terapéuticamente efectiva de un agente quimioterapéutico tal como un inhibidor de formación de tubulina, un inhibidor de topoisomerasa, o un aglutinador de ADN.

Alternativa, o adicionalmente, el segundo compuesto puede ser un anticuerpo que une ErbB2 y bloquea la activación de ligando de un receptor de ErbB. El segundo anticuerpo puede selel anticuerpo monoclonal 2C4 o humanizado 2C4 "Omnitarg" (WO 01/00245). El segundo anticuerpo

puede conjugarse con un agente citotóxico o quimioterapéutico, *por ejemplo*, un maitansinoide, una auristatina, una caliqueamicina, o una fracción de 1,8 bis-naftalimida. Por ejemplo, puede ser deseable proveer además anticuerpos que se unan a EGFR, ErbB2, ErbB3, ErbB4, o factor de crecimiento endotelial vascular (VEGF) en la formulación o régimen de dosificación.

Otros regímenes terapéuticos pueden combinarse con la administración de un agente anti-neoplásico identificado de acuerdo con esta invención. La terapia de combinación puede administrarse como régimen simultáneo o secuencial. Cuando se administra secuencialmente, la combinación puede administrarse en dos o más administraciones. La administración combinada incluye coadministración, usando formulaciones separadas o uan formulación farmacéutica única, y administración consecutiva en cualquier orden, donde se da un período de tiempo, mientras ambos (o todos los) agentes activos ejercen simultáneamente sus actividades biológicas.

En una incorporación, el tratamiento con un ADC de la presente invención involucra la administración combinada de un agente anti-neoplásico identificado en esta solicitud, y uno o más agentes quimioterapéuticos o agentes inhibidores de crecimiento, incluida la coadministración de asociaciones de diferentes agentes quimioterapéuticos, opcionalmente junto con tratamiento con un anticuerpo anti-ErbB2, tal como trastuzumab. Agentes quimioterapéuticos incluyen Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI), taxanos (tales como paclitaxel y doxetaxel) y/o antibióticos de antraciclina. La preparación y pautas posológicas para tales agentes quimioterapéuticos pueden usarse de acuerdo con las instrucciones del fabricante o determinarse empíricamente por el profesional experto. La preparación y pautas posológicas para tal quimioterapia se describen asimismo en Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

El agente antineoplásico puede combinarse con un compuesto anti-hormonal; *por ejemplo*, aun compuesto anti-estrógeno tal como tamoxifeno;

un anti-progesterona tal como onapristona (EP 616812); o un anti-andrógeno tal como flutamida, en posologías conocidas para tales moléculas. Donde el cáncer a tratarse es un cáncer independiente de hormona, el paciente puede haberse sometido previamente a terapia anti-hormonal y, una vez el cáncer se vuelva independiente de hormona, puede administrarse al paciente el anticuerpo anti-ErbB2 (y, opcionalmente, otros agentes, como se describe en esta solicitud). Puede ser también beneficioso coadministrar un cardioprotector (para prevenir o reducir la disfunción del miocardio asociada con la terapia) o una o más citocinas al paciente. Aparte de los anteriores regímenes terapéuticos, el paciente puede someterse a extirpación quirúrgica de las células cancerosas y/o terapia de radiación.

Posologías apropiadas para cualquiera de los anteriores agentes coadministrados son aquellas usadas en la actualidad y pueden disminuirse debido a la acción combinada (sinergia) del agente recientemente identificado y otros agentes quimioterapéuticos o tratamientos.

La terapia de combinación puede producir "sinergia" y probar ser "sinergética", *es decir*, el efecto logrado cuando los ingredientes activos se emplean juntos es mayor que la suma de los efectos que resulta de usar los compuestos separadamente. Un efecto sinergético puede conseguirse cuando los ingredientes activos: (1) se co-formulan y administran o aplican simultáneamente en una formulación farmacéutica unitaria combinada; (2) se aplican alternadamente o en paralelo como formulaciones separadas; o (3) por algún otro régimen. Cuando se aplican en terapia de alternación, puede conseguirse un efecto sinergético cuando los compuestos se administran o aplican secuencialmente, *por ejemplo*, mediante inyecciones diferentes en jeringas separadas. En general, durante la terapia de alternación, una posología efectiva de cada ingrediente activo se administra en forma secuencial, *es decir*, en serie, mientras que en terapia de combinación, posologías efectivas de dos o más ingredientes activos se administran conjuntamente.

METABOLITOS DE LOS CONJUGADOS ANTICUERPO-MEDICAMENTO

También abarcados por el ámbito de esta invención se encuentran los productos metabólicos *in vivo* de los compuestos ADC descritos en esta solicitud, en la medida en que tales productos son novedosos y no obvios con respecto al arte previo. Tales productos pueden resultar, por ejemplo, de la oxidación, reducción, hidrólisis, amidación, esterificación, segmentación enzimática, y similares, del compuesto administrado. Por consiguiente, la invención incluye compuestos novedosos y no obvios, producidos por un proceso que comprende contactar un compuesto de esta invención con un mamífero durante un período de tiempo suficiente para proporcionar un producto metabólico del mismo.

Productos de metabolito pueden identificarse preparando un ADC radiomarcado (*por ejemplo*, ^{14}C ó ^{3}H) ADC, administrándolo parenteralmente en una dosis detectable (*por ejemplo*, superior a alrededor de 0.5 mg/kg) a un animal tal como rata, ratón, conejillo de Indias, mono, o al hombre, dejando suficiente tiempo para que ocurra metabolismo (típicalmente alrededor de 30 segundos a 30 horas) y aislando sus prouctos de conversión de la orina, sangre u otras muestras biológicas. Estos productos se aíslan fácilmente puesto que están marcados (otros se aíslan mediante el empleo de anticuerpos capaces de unir epítopos que sobreviven en el metabolito). Las estructuras de metabolit se determinan de manera convencional, *por ejemplo*, por MS, LC/MS o análisis NMR. En general, el análisis de metabolitos se realiza en la misma forma de los estudios convencionales de metabolismo de medicamentos, bien conocidos por los expertos en el arte. Los productos de conversión, siempre que no se encuentren en caso contrario *in vivo*, son útiles en análisis diagnósticos con respecto a la posología terapéutica de los compuestos ADC.

Metabolitos incluyen los productos de segmentación *in vivo* del ADC, donde ocurre la segmentación de cualquier enlace que une la fracción de medicamento al anticuerpo. Por consiguiente la segmentación metabólica puede resultar en el anticuerpo puro, o un fragmento de

anticuerpo. El metabolito de anticuerpo puede enlazarse a una parte, o la totalidad, del enlazador. La segmentación metabólica puede resultar además en la producción de una fracción de medicamento o parte del mismo. El metabolito de la fracción de medicamento puede unirse a una parte, o la totalidad, del enlazador.

ARTÍCULOS DE FABRICACIÓN

En otra incorporación, se provee un artículo de fabricación, o "kit", que contiene materiales ADC útiles para el tratamiento de los trastornos ya descritos. El artículo de fabricación comprende un envase y una etiqueta o pospecto de envase sobre, o asociado con, el envase. Envases apropiados incluyen, por ejemplo, botellas, frasquitos, jeringas, o envase blíster. Los envases pueden formarse de una variedad de materiales tales como vidrio o plástico. El envase contiene una composición de conjugado anticuerpo-medicamento (ADC), que es efectiva para tratar la enfermedad y puede tener un puerto de acceso estéril (por ejemplo, el envase puede ser una bolsa con solución intravenosa o un frasquito con un tapón perforable por una aguja de inyección hipodérmica). Por lo menos un agente activo en la composición es un ADC. La etiqueta o prospecto de envase indica que la composición se emplea para tratar la enfermedad de elección, tal como cáncer. En una incorporación, la etiqueta o prospecto de envase indica que la composición, que comprende el anticuerpo que une ErbB2, puede usarse para tratar cáncer que expresa un receptor ErbB, seleccioando del grupo conformado por receptor de factor de crecimiento epidérmico (EGFR), ErbB2, ErbB3 y ErbB4. Adicionalmente, la etiqueta o prospecto de envase puede indicar que el paciente a tratarse es uno que tiene cáncer, caracterizado por la activación excesiva de un receptor ErbB seleccionado de EGFR, ErbB2, ErbB3 ó ErbB4. Por ejemplo, el cáncer puede ser uno que sobreexpresa uno de estos receptores y/o que sobreexpresa un ligando ErbB (tal como TGF-α). La etiqueta o prospecto de envase puede también indicar que la composición puede emplearse para tratar cáncer, en donde el cáncer no está caracterizado por sobreexpresión del receptor ErbB2. En

otras incorporaciones, el prospecto de envase puede indicar que la composición ADC puede usarse igualmente para tratar cáncer independiente de hormona, cáncer de próstata, cáncer de colon o cáncer colorrectal.

El artículo de fabricación puede incluir (a) un primer envase con un compuesto contenido en él, en donde el compuesto comprende un ADC de la presente invención, en la cual el anticuerpo del ADC es un primer anticuerpo que une ErbB2 e inhibe el crecimiento de células cancerosas, las cuales sobreexpresan ErbB2; y (b) un segundo envase con un compuesto contenido en él, en donde el compuesto comprende un segundo anticuerpo que une ErbB2 y bloquea la activación de ligando de un receptor ErbB, o un conjugado de este segundo anticuerpo con un maitansinoide. El artículo de fabricación en esta incorporación puede comprender además un prospecto de envase que indica que los compuestos primero y segundo pueden usarse para tratar cáncer. Alternativa, o adicionalmente, el artículo de fabricación puede comprender asimismo un segundo (o tercer) envase que comprende un tampón farmacéuticamente aceptable, tal como agua bacteriostática para inyección (BWFI), salina tamponada con fosfato, solución de Ringery solución de dextrosa. Puede incluir además otros materiales deseables desde un punto de vista comercial y del usuario, incluidos otros tampones, diluyentes, filtros, agujas y jeringas.

EJEMPLOS

Ejemplo 1 – Producción, Caracterización y Humanización del Anticuerpo Monoclonal 4D5 Anti-ErbB2

El anticuerpo monoclonal 4D5 murino, que específicamente une el dominio extracelular de ErbB2, se produjo como se describe en Fendly et al (1990) Cancer Research 50:1550-1558. En pocas palabras, células NIH 3T3/HER2-3_{400} (que expresan aproximadamente 1 x 10 moléculas ErbB2 /célula) producidas como se describe en Hudziak et al (1987) Proc. Natl. Acad. Sci. (USA) 84:7158-7163 se cosecharon con salina tamponada con fosfato (PBS), que contenía 25mM EDTA y se usaron para inmunizar

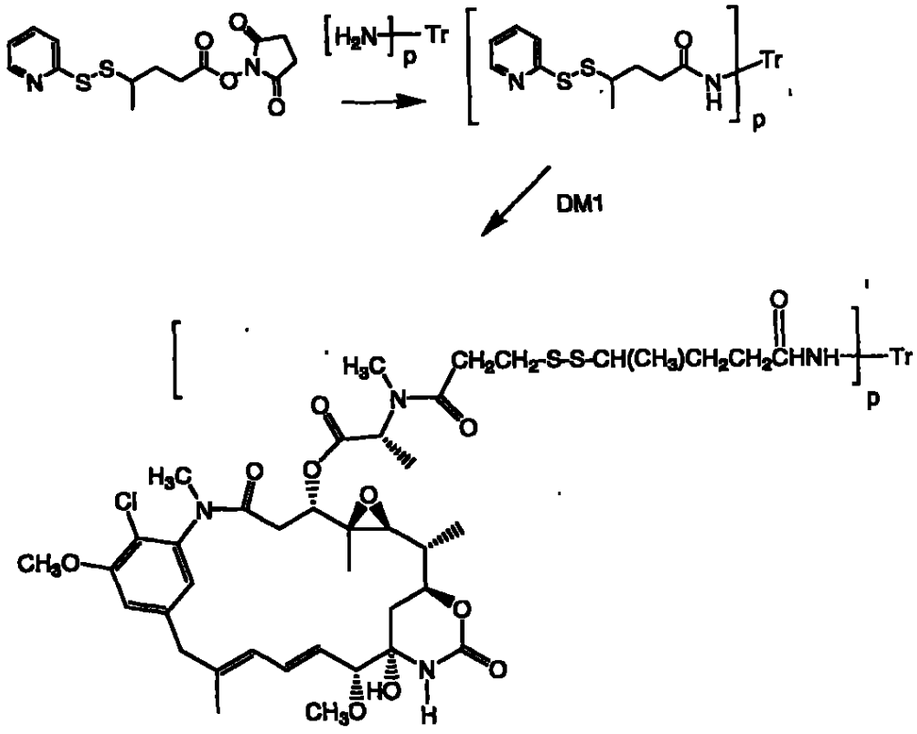
ratones BALB/c. A los ratones se les aplicaron inyecciones i.p. de 10 células en 0.5ml PBS en las semanas 0, 2, 5 y 7. Los ratones con antisueros que inmunoprecipitaron ErbB2 ^{32}P-marcado recibieron inyecciones i.p. de extracto de membrana de ErbB2 purificado en aglutinina de germen de trigo (WGA) – sefarosa, en las semanas 9 y 13. Esto fue seguido por una inyección i.v. de 0.1 ml de la preparación ErbB2, y los esplenocitos se fusionaron con línea de mieloma de ratón X63-Ag8.653. Los sobrenadantes de hibridoma se detectaron selectivamente con respecto a unión ErbB2 mediante ELISA y radioinmunoprecipitación.

El anticuerpo monoclonal 4D5 murino se humanizó usando una estrategia de "mutagénesis de conversión génica", como se describe en US 5821337, cuya completa divulgación se incorpora expresamente por este medio por referencia. El anticuerpo monoclonal 4D5 murino usado en los siguientes experimentos se designa huMAb4D5-8. Este anticuerpo es de isotipo IgG1.

Ejemplo 2 – Purificación de Trastuzumab

Un frasquito que contenía 440 mg HERCEPTIN® (anticuerpo huMAb4D5-8, rhuMAb HER2, US 5821337) se disolvió en 50 mL de tampón MES (25 mM MES, 50 mM NaCl, pH 5.6) y se cargó en una columna de de intercambio catiónico (Sepharose S, 15 cm x 1.7 cm) que se había equilibrado en el mismo tampón. La columna se lavó luego con el mismo tampón (5 volúmenes de columna). Se eluyó trastuzumab aumentando la concentración NaCl del tampón a 200 mM. Se mezclaron fracciones que contenían el anticuerpo, se diluyeron a 10 mg/mL, y se dializaron en un tampón que contenía 50 mm fosfato de potasio, 50 mM NaCl, 2 mM EDTA, pH 6.5.

Ejemplo 3 – Preparación de Conjugados Anticuerpo-Medicamento: Trastuzumab-SPP-DM1:



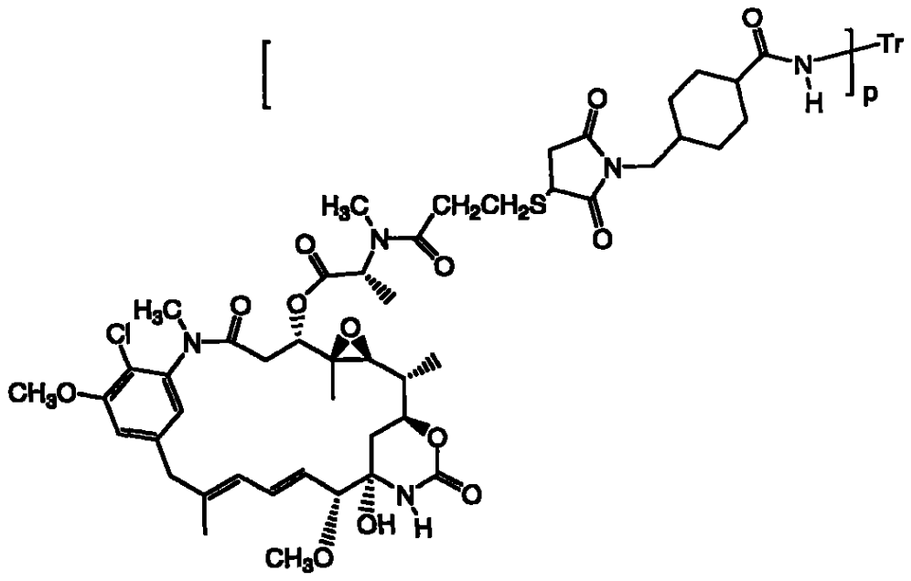
Trastuzumab purificado se derivó con N-succinimidil-4-(2-piridiltio)pentanoato para introducir grupos ditiopiridilo. Trastuzumab (376.0 mg, 8 mg/mL) en 44.7 mL de 50 mM tampón de fosfato de potasio (pH 6.5) que contenía NaCl (50 mM) y EDTA (1 mM) se trató con SPP (5.3 equivalentes molares en 2.3 mL etanol). Luego de incubación durante 90 minutos bajo argón a temperatura ambiente, la mezcla de reacción se filtró en gel a través de una columna Sephadex G25 equilibrada con 35 mM citrato de sodio, 154 mM NaCl, 2 mM EDTA. Se mezclaron y analizaron las fracciones que contenían el anticuerpo. Se determinó el grado de modificación del anticuerpo como se describe anteriormente. La recuperación del anticuerpo modificado (trastuzumab-SPP-Py) fue de 337 mg (89.7%) con 4.5 grupos 2-tiopiridina liberables enlazados por anticuerpo (p').

Trastuzumab-SPP-Py (337.0 mg, 9.5 µmols de grupos 2-tiopiridina liberables) se diluyó con el anterior 35 mM tampón de citrato de sodio, pH 6.5, a una concentración final de 2.5 mg/mL. DM1 ($N^{2'}$-deacetil-$N^{2'}$-(3-mercapto-1-oxopropil)-maitansina), cuya estructura se muestra en Figura 1, (1.7 equivalentes, 16.1 µmoles) en 3.0 mM dimetilacetamida (DMA, 3%

v/v en la mezcla de reacción final) se agregó entonces a la solución de anticuerpo. La reacción avanzó a temperatura ambiente bajo argón durante 20 horas.

La reacción se cargó en una columna de filtración en gel Sephacryl S300 (5.0 cm x 90.0 cm, 1.77 L) equilibrada con 35 mM citrato de sodio, 154 mM NaCl, pH 6.5. La velocidad de flujo fue de 5.0 mL/min y se recogieron 65 fracciones (20.0 mL cada una). Un pico principal se centró alrededor de la fracción No. 47 (Figura 3). El pico principal comprende trastuzumab-SPP-DM1 monomérico. Las fracciones 44-51 se mezclaron y analizaron. El número de moléculas de medicamento DM1 enlazadas por molécula de anticuerpo (p') se determinó midiendo la absorbencia a 252 nm y 280 nm, y se encontró que era de 3.7 moléculas de medicamento por molécula de anticuerpo.

Ejemplo 4 – Preparación de Conjugados Anticuerpo-Medicamento: Trastuzumab-SMCC-DM1



Trastuzumab purificado se derivó con (succinimidil 4-(N-maleimidometil) ciclohexano-1-carboxilato, SMCC, Pierce Biotechnology, Inc) para introducir el enlazador SMCC. Trastuzumab se purificó de

HERCEPTESf®, como en el Ejemplo 2, e intercambio de tampón tratado con 20 mg/mL en 50 mM fosfato de potasio / 50 mM cloruro de sodio / 2 mM EDTA, pH 6.5 con 7.5 a 10 equivalentes molares de SMCC (20 mM en DMSO o DMA (dimetilacetamida), 6.7 mg/mL). Luego de agitar durante 2 a 4 horas bajo argón a temperatura ambiente, la mezcla de reacción se filtró a través de una columna Sephadex G25 equilibrada con 50mM fosfato de potasio / 50 mM cloruro de sodio / 2 mM EDTA, pH 6.5. Alternativamente, la mezcla de reacción se filtró en gel con 30 mM citrato de sodio y 150 mM cloruro de sodio a pH 6. Se mezclaron y analizaron las fracciones que contenían el anticuerpo. La recuperación de trastuzumab-SMCC fue de 88%.

El intermedio medicamento-enlazador, trastuzumab-SMCC de antes, se diluyó con 50 mM fosfato de potasio / 50 mM cloruro de sodio / 2 mM EDTA, pH 6.5, a una concentración final de 10 mg/ml, y se hizo reaccionar con una solución 10 mM de DM1 (1.7 equivalentes asumiendo 5 SMCC/trastuzumab, 7.37 mg/ml) en dimetilacetamida. La reacción se agitó a temperatura ambiente bajo argón durante 4 a alrededor de 16 horas. La mezcla de reacción de conjugación se filtró a través de una columna de filtración en gel Sephadex G25 (1.5 x 4.9 cm) con 1 x PBS a pH 6.5. Alternativamente, la mezcla de reacción se filtró en gel con 10 mM succinato y 150 mM cloruro de sodio a pH 5. La relación DM1/trastuzumab (p) fue de 3.1, medido por la absorbencia a 252 nm y a 280 nm. La relación de medicamento a anticuerpo (p) puede medirse también por espectrometría de masa. Asimismo, la conjugación puede monitorearse por electroforesis en gel de poliacrilamida SDS. La agregación puede evaluarse mediante análisis de dispersión luminosa de láser.

Conjugados Anticuerpo-SMCC-DM1:

Siguiendo este protocolo, se prepararon otros conjugados anticuerpo-medicamento con el enlazador SMCC y la fracción de medicamento DM1, incluyendo:

Conjugado anticuerpo-medicamento (ADC)	Anticuerpo (Ab)	Antígeno	Carga media de medicamento (DM1/Ab)
10H-SMCC-DM1	10H1 (murino) anti-NaPi3b	NaPi3b	5.0
2H9-SMCC-DM1	2H9 Mab	anti-EphB2R	4.0
ragweed-SMCC-DM1	ragweed		2.7
antiTENB2-SMCC-DM1	anti-TENB2-3416#2 de ratón MAb	TENB2	2.5
anti-TENB2 20D1 Fc Chimera-SMCC-DM1	anti-TENB2 de ratón, humano quimérico	TENB2	3.32
antiCD19-SMCC-DM1	antiCD19 (mIgG1 isotipo)	CD19	5.05
2H7-SMCC-DM1	antiCD20 (mIgG2a isotipo)	CD20	2.44, 3.84 (dos lotes)
CD20 LC-SMCC-DM1	antiCD20 (Rituxan®)	CD20	
HB5-SMCC-DM1	antiCD21 (mIgG2a isotipo)	CD21	4.05
RFB4-SMCC-DM1	antiCD22	CD22	1.95-6.75 (múltiples lotes)
Cy34.1.2-SMCC-DM1	antiCD22	CD22	2.70
12F7.1.5-SMCC-DM1	antiCD22	CD22	5.90
9A8.1.1-SMCC-DM1	antiCD22	CD22	5.00
8C9.1.2-SMCC-DM1	antiCD22	CD22	2.90
14B3.3.1-SMCC-DM1	antiCD22	CD22	5.20
8G10.4.2-SMCC-DM1	antiCD22	CD22	3.60
2B4.1.4-SMCC-DM1	antiCD22	CD22	5.20
7A2.4.1-SMCC-DM1	antiCD22	CD22	3.60
4H3.2.2-SMCC-DM1	antiCD22	CD22	3.60
5E8.1.8-SMCC-DM1	antiCD22	CD22	3.60
3F11.2.1-SMCC-DM1	antiCD22	CD22	4.50
6C9.1.3-SMCC-DM1	antiCD22	CD22	3.70
10D2.4.3-SMCC-DM1	antiCD22	CD22	3.65
10D6.8.1-SMCC-DM1	antiCD72	CD72	2.90
ZL7-SMCC-DM1	antiCD79a (alfa)	CD79a	3.60
5C3-SMCC-DM1	antiCD79a (alfa)	CD79a	1.85
6G1-SMCC-DM1	antiCD79a (alfa)	CD79a	3.50
7H7-SMCC-DM1	antiCD79a (alfa)	CD79a	3.25
8D11-SMCC-DM1	antiCD79a (alfa)	CD79a	3.60

8H9-SMCC-DM1	antiCD79a (alfa)	CD79a	3.40
11E5-SMCC-DM1	antiCD79a (alfa)	CD79a	3.60
15E4-SMCC-DM1	antiCD79a (alfa)	CD79a	3.25
16C11-SMCC-DM1	antiCD79a (alfa)	CD79a	2.50
2F2-SMCC-DM1	antiCD79b (beta)	CD79b	3.20
17A7-SMCC-DM1	antiCD79b (beta)	CD79b	4.40
SN8-SMCC-DM1	antiCD79b (beta)	CD79b	4.40, 0.80 (dos lotes)
1F9-SMCC-DM1	antiFcRH1	FcRH1	4.20
7A2-SMCC-DM1	antiFcRH2	FcRH2	4.20, 3.95 (dos lotes)
7G7-SMCC-DM1	antiFcRH2	FcRH2	3.80
Cl1D6-SMCC-DM1	antiFcRH2	FcRH2	2.20
51505.111-SMCC-DM1	antiCXCR5	CXCR5	6.10
12C7 Fc Chimera-SMCC-DM1	antiBrevican	brevican	4.60
H2:PSCA hlog-SMCC-DM1	antiPSCA hlog	PSCA	3
H6:xPSCAhlog-SMCC-DM1	antiPSCA hlog	PSCA	2.6
xFcRH5:3909 7D11.1.1-SMCC-DM1	antiIRTA2	IRTA2	2.6
xFCRH5.7D11-SMCC-DM1	antiIRTA2	IRTA2	4.4
11D10LC-SMCC-DM1	anti-CA 0772P de ratón MAb	CA 0772P MUC16	
xNCA LC-SMCC-DM1	anti-CEACAM6 de ratón MAb	CEACAM6 NCA	

Conjugados Anticuerpo-BMPEO-DM1:

Para conjugación de cisteína, el anticuerpo puede reducirse con un agente reductor tal como ditiotreitol (DTT) o TCEP, en la presencia de EDTA. Luego de incubar durante alrededor de una hora a 37°C, el pH se ajusta a alrededor de 7 con 100 mM fosfato de potasio. El anticuerpo reducido se modifica por el reactivo bis-maleimido BM(PEO)4 (Pierce Chemical), dejando un grupo maleimido sin reaccionar en la superficie del anticuerpo. Esto puede realizarse disolviendo BM(PEO)4 en una mezcla al 50% de etanol/agua a una concentración de 10 mM y agregando un exceso molar de diez veces a una solución, que contiene anticuerpo en solución salina tamponada con fosfato a una concentración de aproximadamente 1.6

mg/ml (10 μM), dejándolo reaccionar durante 1 hora para formar Ab-BMPEO. Se retira el exceso de BM(PEO)4 por filtración en gel (columna HiTrap, Pharmacia) en 30 mM citrato, pH 6 con 150 mM tampón NaCl. Un exceso molar de 10 veces, aproximado, DM1 se disuelve en dimetil acetamida (DMA) y se agrega al intermedio Ab-BMPEO. Igualmente puede emplearse dimetil formamida (DMF) para disolver el reactivo de la fracción de medicamento. Se deja reaccioanr la mezcla de reacción de un día para otro antes de la filtración en gel o diálisis en PBS para retirar el DM1 sin reaccioanr. Se usó la filtración en gel en columnas S200 en PBS para retirar los agregados de alto peso molecular y proporcionar Ab-BMPEO-DM1 purificado.

Siguiendo este protocolo, se prepararon otros conjugados anticuerpo-medicamento con el enlazador BMPEO y la fracción de medicamento DM1, incluidos:

Conjugado anticuerpo-medicamento (ADC)	Anticuerpo (Ab)	Antígeno	Carga media de medicamento (DM1/Ab)
trastuzumab-BM(PEO)-DM1	trastuzumab (Herceptin®)	HER2	2.94
CD120-BMPEO4-DM1	anti-GP120 de ratón MAb	GP120	2
RFB4-BMPEC4-DM1	antiCD22	CD22	3.7 y 4.25 (dos lotes)

Ejemplo 5 – Análisis de Proliferación In Vitro

Se midió la eficacia de ADC mediante un análisis de proliferación empleando el siguiente protocolo (Promega Corp. Technical Bulletin TB288; Mendoza et al (2002) Cancer Res. 62:5485-5488):

1. Una alícuota de 100 μl de cultivo de células que contenía alrededor de 10^4 células (SKBR-3, BT474, MCF7 ó MDA-MB-468) en medio se depositó en cada pocillo de una placa de pared opaca, de 96 pocillos.

2. Se prepararon pocillos de control, que contenían medio, y sin células.
3. Se agregó ADC a los pocillos experimentales y se incubó durante 3-5 días.
4. Las placas se equilibraron a temperatura de la sala durante aproximadamente 30 minutos.
5. Se agregó un volumen de reactivo CellTiter-Glo igual al volumen de medio de cultivo celular presente en cada pocillo.
6. Se mezclaron los contenidos durante 2 minutos en un agitador orbital para inducir lisis de las células.
7. La placa se incubó a temperatura de la sala durante 10 minutos para estabilizar la señal de luminescencia.
8. Se registró la luminescencia y se presentó en gráficos como RLU = unidades de luminiscencia relativa.

Ejemplo 6 – Depuración Sérica y Estabilidad en Ratones

Se estudiaron ratones beige no sometidos previamente a experimentación (sin tumores), lampiños, en seis grupos de cuatro animales. El día 0, cada ratón recibió una dosis única 2 mg/kg de ADC en 200 µl de portador acuoso, excepto el grupo vehículo, que recibió únicamente portador. Se recogió sangre mediante punción cardiaca bajo anestesia en cada punto de tiempo (5 minutos, 1 hora, 6 horas, 24 horas, 72 horas, y 168 horas) pos-dosis. Se aisló el suero y se midió el anticuerpo y ADC.

Grupo 1: Vehículo (PBS, pH 6.5, sin ADC)

Grupo 2: Trastuzumab-SMCC-DM1

Grupo 3: Trastuzumab-SPP-DM1

Grupo 4: Trastuzumab-SPDP-DM1

Grupo 5: Trastuzumab-SPP-DM3

Grupo 5: Trastuzumab-SPP-DM4

Ejemplo 7 – Estabilidad Sérica en Ratas

Se estudiaron seis grupos de dosis con 6 ratas Sprague-Dawley (100-125 gms cada una) por dosis. El día 0, se administró a los animales una dosis única IV de vehículo, 10 mg/kg trastuzumab-SPP-DM1, ó 10 mg/kg trastuzumab-SMCC-DM1, en un volumen de dosis de 10 ml/kg a través de la vena lateral de la cola. Aproximadamente 300 µl de sangre entera se recogieron en cada punto de tiempo: 0 (dosis previa), 10, y 30 minutos; 1, 2, 4, 8, 24 y 36 horas; y 2, 3, 4, 7, 14, 21, 28 días pos-dosis.

Ejemplo 8 – Eficacia *In Vivo* sobre el Volumen del Tumor

Ratones con explante transgénico de alta expresión de HER2:

Animales apropiados para experimentos transgénicos pueden obtenerse de fuentes comerciales estándar tales como Taconic (Germantown, N. Y.). Son apropiadas muchas cepas, pero se prefieren ratones hembra FVB debido a su susceptibilidad más elevada a la formación de tumores. Se emplearon machos FVB para el apareamiento y se utilizaron sementales CD.1 vasectomizados para estimular la pseudopreñez. Ratones vasectomizados pueden obtenerse de cualquier proveedor comercial. Los fundadores se cruzaron con ratones FVB o con ratones heterocigotos 129/BL6 x FVB p53. Los ratones con heterocigocidad en el alelo p53 se usaron para aumentar potencialmente la formación de tumores. Sin embargo, se demostró que esto era innecesario. Por consiguiente, algunos tumores F1 son de cepa mixta. Tumores de fundadores son FVB únicamente. Se obtuvieron seis fundadores con algunos tumores en desarrollo sin tener camadas.

Los animales que tenían tumores (aloinjerto propagado de ratones transgénicos Fo5 mmtv) se trataron con una sola inyección de un conjugado maitansinoide trastuzumab-DM1 (dosis 10 mg/kg) y se evaluó el volumen del tumor durante 20 días después de la inyección.

Ratones SCID con Xenoinjerto Bjab-luc:

A grupos de ocho a diez ratones SCID con 20 millones de células tumorales de xenoinjerto Bjab-luc por ratón se administró el día 1 un conjugado anticuerpo-medicamento, o un anticuerpo puro. Se probó un grupo de ocho ratones cada uno con ADC anti-CD22, anticuerpos anti-CD22 puros, y control. ADC de control (trastuzumab-SMCC-DM1; carga: DM1/trastuzumab =3.2) no es un aglutinante específico y se administró a 200 μg DM1/m2, 4.2 trastuzumab/kg ratón, y resultó en un tiempo medio de duplicación del tumor de alrededor de 3 días. Conjugados anti-CD22 7A2-SMCC-DM1 (carga: DM1/Ab = 3.6), 5E8-SMCC- DM1 (carga: DM1/Ab = 3.6), y RFB4-SMCC-DM1 (carga: DM1/Ab = 4.3). Anticuerpos puros, 7A2, 5E8, y RFB4 se dosificaron a 4 mg/kg ratón.

Ejemplo 9 – Toxicidad en Rata

Se evaluó el perfil de toxicidad aguda de trastuzumab-SPP-DM1 (en lazador disulfuro) comparado con el de DM1 libre y trastuzumab-SMCC-DM1 (enlazador no disulfuro). Los animales se inyectaron el día 1, se obtuvieron perfiles completos de química y hematología en la línea de referencia, el día 3 y el día 5, y se realizó una necropsia completa el día 5. Se llevó a cabo histología de rutina en tres animales aleatorios por cada grupo para los siguientes tejidos: esternón, hígado, riñón, timo, bazo, intestino grueso y delgado. Los grupos experimentales fueron los siguientes:

Grupo 1: Vehículo (10 mM succinato de sodio, 100 mg/mL sacarosa, 0.1% Tween 20, pH 5.0)

Grupo 2: Trastuzumab-SPP-DM1, 22.3mg/kg

Grupo 3: Trastuzumab-SMCC-DM1, 10mg/kg

Grupo 4: Trastuzumab-SMCC-DM1, 25mg/kg

Grupo 5: Trastuzumab-SMCC-DM1, 50mg/kg

Grupo 6: Free DM1, 160μg/kg

Ejemplo 10 – Toxicidad/Seguridad en Mono Cynomolgus

Se estudiaron tres grupos de cuatro (2 machos, 2 hembras) de *Macaca fascicularis* (mono cynomolgus) no sometidos previamente a experimentación.

Grupo 1: (4 animales) recibieron únicamente vehículo (PBS, pH 6.5, *es decir*, formulación sin ADC) el día 1 y el día 22, seguido por necropsia el día 36.

Grupo 2: (4 animales) recibieron trastuzumab-SMCC-DM1 4900 $\mu g/m^2$ el día 1 y el día 22.

Grupo 3: (4 animales) recibieron trastuzumab-SMCC-DM1 7200 $\mu g/m^2$ el día 22.

La dosis se expresa en área de superficie de un animal, de manera que sea relevante a otras especies, *es decir*, la dosificación de $\mu g/m^2$ es independiente de la especie y, por lo tanto, comparable entre especies. Formulaciones de trastuzumab-SMCC-DM1 para estudios del Grupo 2 y el Grupo 3 contenían PBS, 5.4mM fosfato de sodio, 4.2 mM fosfato de potasio, 140 mM cloruro de sodio, pH 6.5.

Se recogió sangre para análisis de hematología antes de la primera dosificación (período de aclimatación) y los días 3, 7, 11, y 14 luego de la primera dosis (Grupos 1 y 2) y los días 3, 7, 11, 14, y 21 después de la segunda dosis (Grupos 1, 2 y 3). Los conteos de eritrocitos (RBC) y plaquetas (PLT) se midieron por el método de dispersión luminosa. El conteo de leucocitos (WBC) se midió por el método peroxidasa/basófilo. El conteo de reticulocitos se midió por el método de dispersión luminosa con tinte catiónico. Los conteos de células se midieron en un aparato Advia 120. ALT (alanina aminotransferasa) y AST (aspartato aminotransferasa) se midieron en U/L por UV/NADH; metodología IFCC en un aparato Olympus AU400, y usando pruebas Total Ab ELISA - ECD/GxhuFc-HRP. Conj. Ab ELISA - xDM1/ECD-Bio/SA-HRP.

Todas las patentes, solicitudes de patente y referencias citadas de principio a fin de la expecificación se incorporan expresamente por referencia.

Reivindicamos:

1. Un compuesto de conjugado anticuerpo-medicamento (ADC), que comprende un anticuerpo unido covalentemente por un enlazador a una o más fracciones de medicamento maitansinoide, compuesto que tiene la Fórmula I:

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

o su sal farmacéuticamente aceptable o solvato, en donde:

Ab es un anticuerpo que se une a un receptor ErbB, o que se une a uno o más antígenos asociados a un tumor o receptores de superficie celular seleccionados de (1)-(36):

(1) BMPR1B (proteína morfogenética ósea tipo receptor IB, No. Acceso a Genbank NM_001203);

(2) E16 (LAT1, SLC7A5, No. Acceso a Genbank NM_003486);

(3) STEAP1 (antígeno epitelial de transmembrana de seis hélices de próstata, No. Acceso a Genbank NM_012449);

(4) 0772P (CA125, MUC16, No. Acceso a Genbank AF361486);

(5) MPF (MPF, MSLN, SMR, factor potenciador de megacariocito, mesotelina, No. Acceso a Genbank NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, familia portadora de soluto 34 (fosfato de sodio), miembro 2, transportador 3b de fosfato dependiente de sodio tipo II, No. Acceso a Genbank NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaforina 5b Hlog, dominio sema, siete repeticiones de trombospondina (tipo 1 y similar a tipo 1), dominio de transmembrana (TM) y dominio citoplásmico corto, (semaforina) 5B, No. Acceso a Genbank AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, No. Acceso a Genbank AY358628);

(9) ETBR (receptor tipo B de endotelina, No. Acceso a Genbank AY275463);

(10) MSG783 (RNF124, proteína hipotética FLJ20315, No. Acceso a Genbank NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, gen 1 asociado con cáncer de próstata, proteína 1 asociada con cáncer de próstata, antígeno epitelial de membrana de seis hélices de próstata 2, proteína de próstata de transmembrana de seis hélices, No. Acceso a Genbank AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, canal de catión potencial de receptor transitorio, subfamilia M, miembro 4, No. Acceso a Genbank NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, factor de crecimiento derivado de teratocarcinoma, No. Acceso a Genbank NP_003203 ó NM_003212);

(14) CD21 (CR2 (receptor 2 de Complemento) o C3DR (receptor del virus C3d/Epstein Barr) o Hs.73792 No. Acceso a Genbank M26004);

(15) CD79b (CD79B, CD79β, IGb (beta asociada a inmunoglobulina), B29, No. Acceso a Genbank NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (dominio SH2 que contiene proteína 1a de anclaje fosfatasa), SPAP1B, SPAP1C, No. Acceso a Genbank NM_030764);

(17) HER2 (No. Acceso a Genbank M11730);

(18) NCA (No. Acceso a Genbank M18728);

(19) MDP (No. Acceso a Genbank BC017023);

(20) IL20Rα (No. Acceso a Genbank AF184971);

(21) Brevican (No. Acceso a Genbank AF229053);

(22) EphB2R (No. Acceso a Genbank NM_004442);

(23) ASLG659 (No. Acceso a Genbank AX092328);

(24) PSCA (No. Acceso a Genbank AJ297436);

(25) GEDA (No. Acceso a Genbank AY260763;

(26) BAFF-R (receptor de factor activador de linfocito B, receptor BLyS 3, BR3, NP_443177.1);

(27) CD22 (isoforma CD22-B de receptor de linfocito B, NP-001762.1);

(28) CD79a (CD79A, CD79α, alfa asociada a inmunoglobulina, una proteína específica de linfocito B que interactúa covalentemente con Ig beta (CD79B) y forma un complejo en la superficie con moléculas IgM, transduce una señal involucrada en la diferenciación de linfocitos B, No. Acceso a Genbank NP_001774.1);

(29) CXCR5 (receptor 1 de linfoma 1 de Burkitt, un receptor acoplado a proteína G, que se activa por la quimiocina CXCL13, funciona en migración de linfocitos y defensa humoral, juega un papel en la infección HIV-2 y, quizá, desarrollo de SIDA, linfoma, mieloma, y leucemia, No. Acceso a Genbank NP_001707.1);

(30) HLA-DOB (subunidad Beta de la molécula clase II MHC (antígeno la), que une péptidos y los presenta a linfocitos T CD4+, No. Acceso a Genbank NP_002111.1);

(31) P2X5 (canal iónico 5, abierto por acción de ligando P2X, de receptor purinérgico, un canal iónico abierto por ATP extracelular, puede estar involucrado en la transmisión sináptica y neurogénesis; la deficiencia puede contribuir a la patofisiología de inestabilidad detrusora idiopática, No. Acceso a Genbank NP_002552.2);

(32) CD72 (antígeno CD72 de diferenciación de linfocitos B, Lyb-2, No. Acceso a Genbank NP_001773.1);

(33) LY64 (antígeno 64 de linfocitos (RP105), proteína de membrana tipo I de la familia de repeticiones rica en leucina (LRR), regula la activación y apoptosis de linfocitos B; la pérdida de función se asocia con actividad patológica incrementada en pacientes con lupus sistémico eritematoso, No. Acceso a Genbank NP_005573.1);

(34) FcRH1 (proteína 1 similar a receptor Fc, un receptor putativo para el dominio Fc de inmunoglobulina que contiene dominios similares a

Ig tipo C2 y dominios ITAM, puede tener un papel en la diferenciación de linfocitos B, No. Acceso a Genbank NP_443170.1);

(35) IRTA2 (asociado 2 de translocación de receptor de superfamilia de inmunoglobulina, un inmunorreceptor putativo con posibles papeles en el desarrollo de linfocitos B y génesis de linfoma; la desregulación del gen por translocación ocurre en algunas neoplasias malignas de linfocitos B, No. Acceso a Genbank NP_112571.1); y

(36) TENB2 (proteoglucano de transmembrana putativo, relacionado con la familia EGF/herregulina de factores de crecimiento y folistatina, No. Acceso a Genbank AF179274;

siempre que el anticuerpo no sea TA.1.

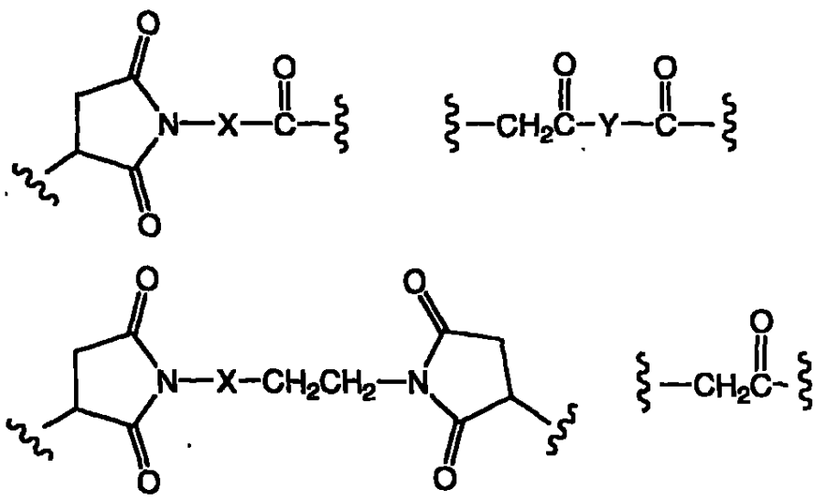
L es un enlazador no disulfuro.

D es una fracción de medicamento maitansinoide; y

p es 1 a 8.

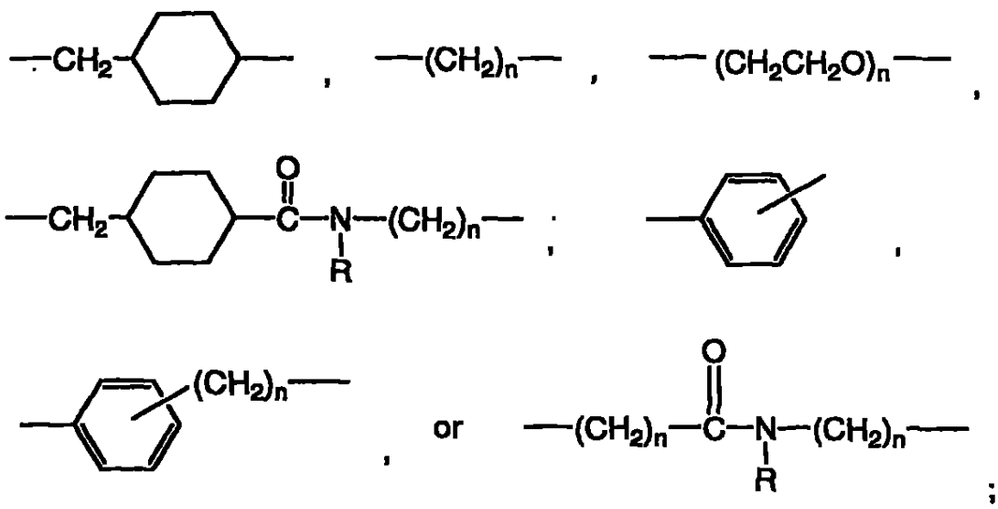
2. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 en donde Ab es un anticuerpo que se une a uno o más antígenos asociados con tumores o receptores de superficie celular seleccionados de (1)-(16) y (18)-(36).

3. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 o la reivindicación 2 en donde L es un enlazador seleccionado de las estructuras:

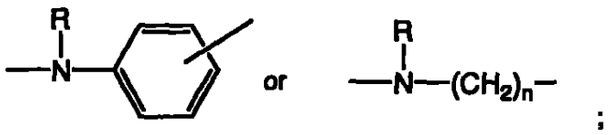


donde las líneas onduladas indican las uniones covalentes a Ab y D;

X es:

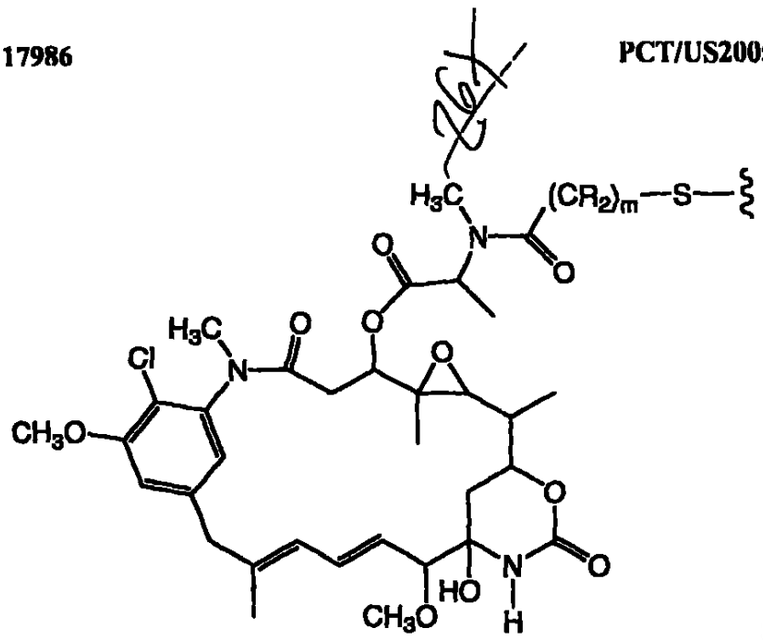


Y es:



R es independientemente H ó C_1-C_6 alquilo; y n es 1 a 12;

4. El compuesto de conjugado anticuerpo-medicamento de cualquiera de las reivindicaciones precedentes en donde D se selecciona de la estructura:



;

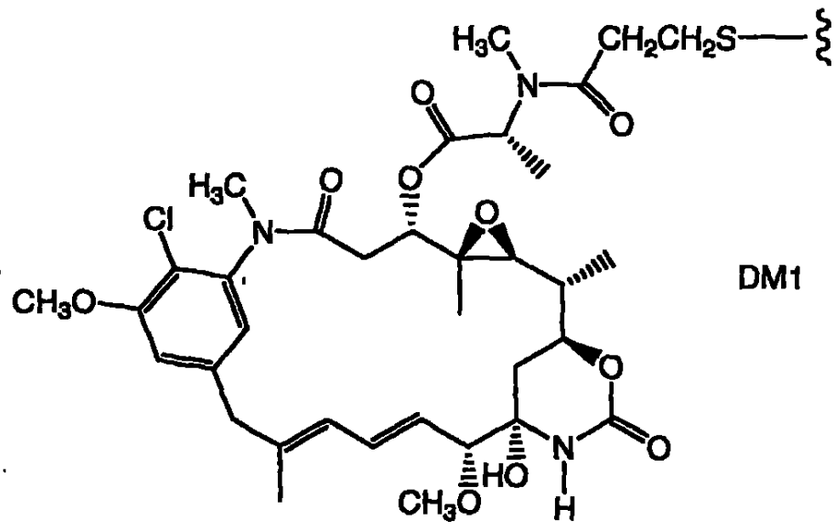
en donde la línea ondulada indica la unión covalente a L;

R es independientemente H ó C_1-C_6 alquilo; y

m es 1, 2, ó 3.

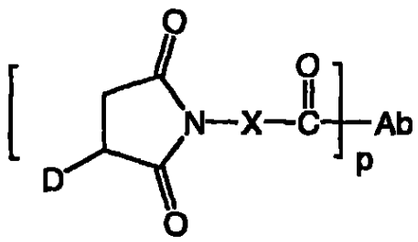
5. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 4 en donde m es 2, y R es H.

6. El compuesto de conjugado anticuerpo-medicamento de cualquiera de las reivindicaciones precedentes en donde la fracción de medicamento maitansinoide es DM1, que tiene la estructura:

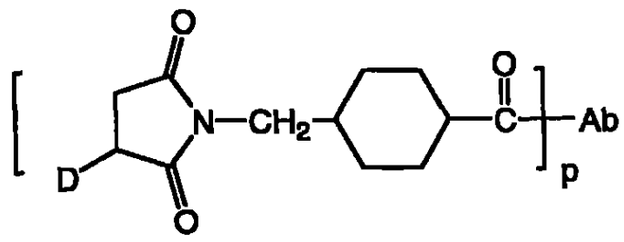


DM1

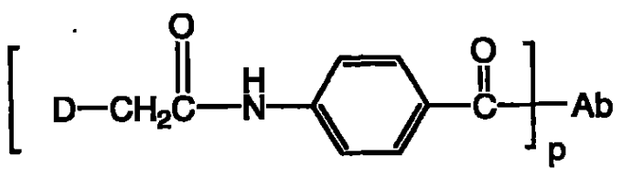
7. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 3 que tiene la estructura:



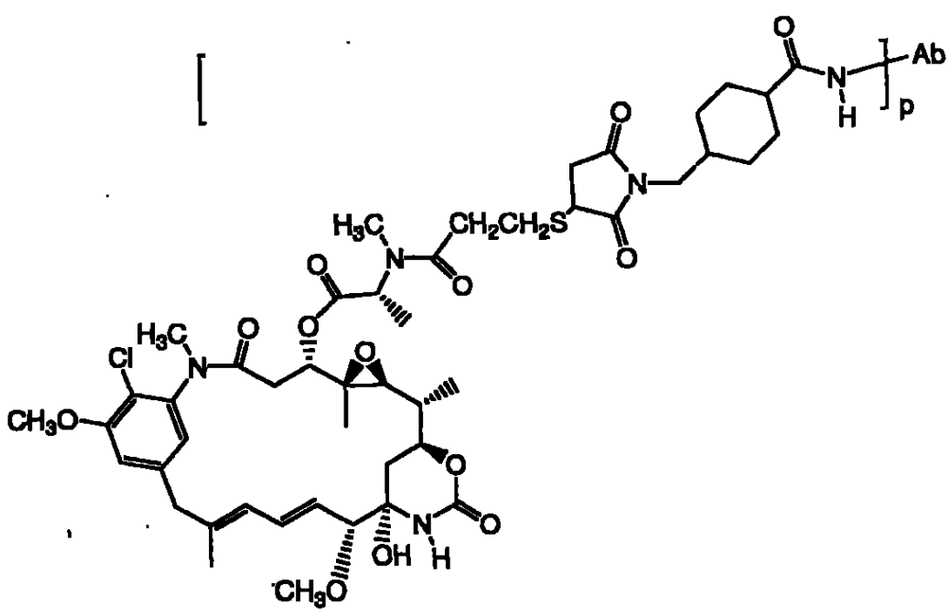
8. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 7 que tiene la estructura:



9. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 3 que tiene la estructura:

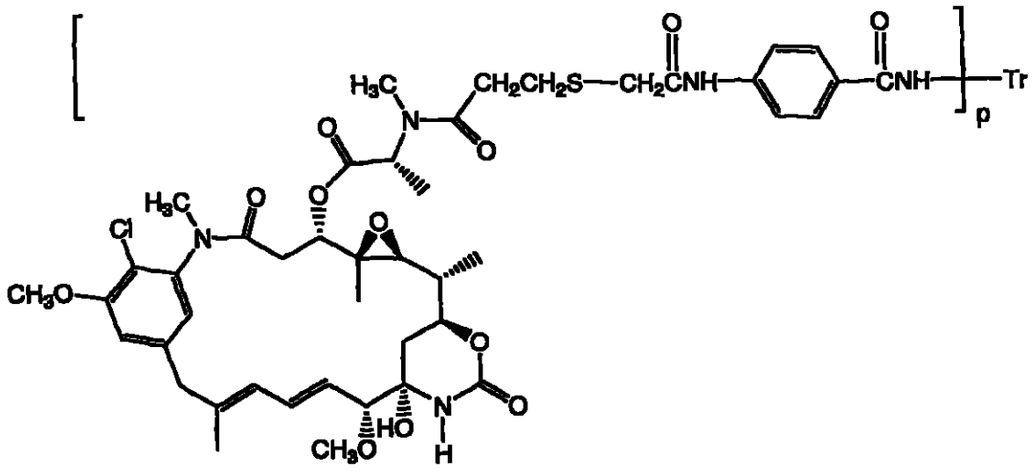


10. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 que tiene la estructura:



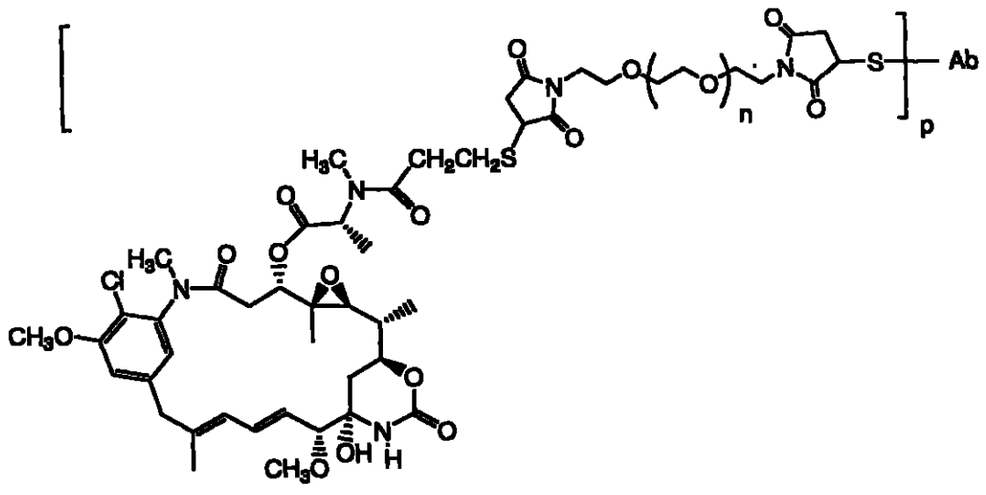
11. El conjugado anticuerpo-medicamento de la reivindicación 10 en donde Ab es trastuzumab, y p es 1, 2, 3, ó 4.

12. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 que tiene la estructura:



donde Tr es trastuzumab, y p es 1, 2, 3, ó 4.

13. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 que tiene la estructura:



en donde n es 0, 1, ó 2; y p es 1, 2, 3, ó 4.

14. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 13 en donde Ab es trastuzumab.

15. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 en donde p es 1, 2, 3, ó 4.

16. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 en donde el anticuerpo se une a un receptor codificado por un gen ErbB.

17. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 16 en donde el receptor se selecciona de EGPR, HER2, HER3 y HER4.

18. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 17 en donde el anticuerpo se une específicamente a un receptor HER2.

19. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 que se une específicamente al dominio extracelular del receptor HER2 e inhibe el crecimiento de células tumorales, que sobreexpresan el receptor HER2.

20. El compuesto de conjugado anticuerpo-medicamento de cualquiera de las reivindicaciones precedentes en donde el anticuerpo se selecciona de un anticuerpo monoclonal, un fragmento de anticuerpo, un anticuerpo quimérico, y un anticuerpo humanizado.

21. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 en donde el anticuerpo es un anticuerpo humanizado seleccionado de huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 y huMAb4D5-8 (trastuzumab).

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22. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 21 en donde el anticuerpo es huMAb4D5-8 (trastuzumab).

23. El conjugado anticuerpo-medicamento de la reivindicación 20 en donde el anticuerpo es un fragmento Fab.

24. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 en donde el anticuerpo se une al enlazador a través de un tiol de cisteína del anticuerpo.

25. El compuesto de conjugado anticuerpo-medicamento de la reivindicación 24 en donde p es 1, 2, 3, ó 4.

26. Una composición farmacéutica que comprende el compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 o reivindicación 2, o su sal farmacéuticamente aceptable, y un diluyente, portador o excipiente farmacéuticamente aceptable.

27. La composición farmacéutica de la reivindicación 26, que comprende además una cantidad terapéuticamente efectiva de un agente quimioterapéutico seleccionado de Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozol, Imatinib mesilato, PTK787/ZK 222584, Oxaliplatino, 5-FU, Leucovorin, Rapamicina, Lapatinib, Lonafarnib, Sorafenib, y Gefitinib.

28. La composición farmacéutica de la reivindicación 26, que comprende además una cantidad terapéuticamente efectiva de un agente anti-angiogénico.

29. La composición farmacéutica de la reivindicación 26, que comprende además una cantidad terapéuticamente efectiva de bevacizumab.

30. Un método de inhibir la proliferación celular, que comprende tratar células de mamífero en un medio de cultivo celular con un compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 ó la reivindicación 2, con lo cual se inhibe la proliferación de las células.

31. El método de la reivindicación 30 en donde las células de mamífero tienen proteínas de receptor HER2 al compuesto de conjugado anticuerpo-medicamento.

32. El método de la reivindicación 31 en donde las células de mamífero son células tumorales de mama.

33. El método de la reivindicación 30 en donde el compuesto de conjugado anticuerpo-medicamento es más citotóxico que un compuesto maitansinoide que comprende la fracción maitansinoide del compuesto de conjugado anticuerpo-medicamento.

34. El método de la reivindicación 30 en donde el conjugado anticuerpo-medicamento induce apoptosis.

35. Un método de tratar cáncer, que comprende administrar a un paciente una formulación de un de un compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 ó la reivindicación 2, y un diluyente, portador o excipiente farmacéuticamente aceptable.

36. El método de la reivindicación 35, en donde el cáncer se selecciona del grupo conformado por cáncer de mama, ovario, estómago, endometrio, glándula salival, pulmón, riñón, colon, colorrectal, tiroides, páncreas, próstata y vejiga.

37. El método de la reivindicación 36, en donde el cáncer es cáncer de mama que sobreexpresa ErbB2 en un nivel 2+ o más.

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38. El método de la reivindicación 35, en donde la cantidad de compuesto de conjugado anticuerpo-medicamento administrada al paciente está en el rango de alrededor de 0.1 a alrededor de 10 mg/kg de peso del paciente por dosis.

39. El método de la reivindicación 35, en donde el conjugado anticuerpo-medicamento se administra en intervalos de alrededor de tres semanas.

40. El método de la reivindicación 35, en donde el conjugado anticuerpo-medicamento se administra por infusión.

41. El método de la reivindicación 35, en donde el conjugado anticuerpo-medicamento se formula con un vehículo parenteral farmacéuticamente aceptable.

42. El método de la reivindicación 41, en donde el conjugado anticuerpo-medicamento se formula en una forma inyectable de posología unitaria.

43. El método de la reivindicación 42, en donde el conjugado anticuerpo-medicamento se administra intravenosamente.

44. El método de la reivindicación 35, en donde al paciente se le administra un anticuerpo inhibidor de crecimiento, en combinación con el compuesto de conjugado anticuerpo-medicamento.

45. El método de la reivindicación 35, en donde al paciente se le administra un segundo anticuerpo, que une ErbB2 y bloquea la activación de ligando de un receptor ErbB, en combinación con el compuesto de conjugado anticuerpo-medicamento.

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46. El método de la reivindicación 45, en donde el segundo anticuerpo comprende anticuerpo monoclonal 2C4 ó humanizado 2C4.

47. El método de la reivindicación 46, en donde dicho segundo anticuerpo se conjuga con un agente citotóxico.

48. El método de la reivindicación 35, en donde al paciente se le administra un agente quimioterapéutico, en combinación con el compuesto de conjugado anticuerpo-medicamento, donde el agente quimioterapéutico se selecciona de Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozol, Imatinib mesilato, PTK787/2K 222584, Oxaliplatino, 5-FU, Leucovorin, Rapamicina, Lapatinib, Lonafarnib, Sorafenib, y Gefitinib.

49. El método de la reivindicación 35, en donde al paciente se le administra un agente anti-angiogénico en combinación con el compuesto de conjugado anticuerpo-medicamento, donde el agente anti-angiogénico se selecciona de bevacizumab.

50. Un método de inhibir el crecimiento de células tumorales que sobreexpresan un receptor de factor de crecimiento, que comprende administrar a un paciente un conjugado anticuerpo-medicamento de la reivindicación 1, que se une específicamente a un receptor de factor de crecimiento seleccionado de HER2 ó EGF y un agente quimioterapéutico, en donde dicho conjugado anticuerpo-medicamento y dicho agente quimioterapéutico se administran cada uno en cantidades efectivas para inhibir el crecimiento de células tumorales en el paciente.

51. Un método para el tratamiento de un paciente humano susceptible a, o diagnosticado con, un trastorno caracterizado por sobreexpresión del receptor ErbB2, que comprende administrar una

combinación de un conjugado anticuerpo-medicamento de la reivindicación 1 y un agente quimioterapéutico o un agente inhibidor de crecimiento.

52. El método de la reivindicación 51, en donde el anticuerpo del conjugado anticuerpo-medicamento es un anticuerpo anti-ErbB2.

53. El método de la reivindicación 52, en donde el anticuerpo anti-ErbB2 tiene una característica biológica de un anticuerpo monoclonal 4D5.

54. El método de la reivindicación 52m en donde el anticuerpo anti-ErbB2 une esencialmente el mismo epítopo que el anticuerpo monoclonal 4D5.

55. El método de la reivindicación 52, en donde el anticuerpo anti-ErbB2 se selecciona del grupo conformado por anticuerpos humanizados huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 y huMAb4D5-8 (trastuzumab).

56. Un análisis para detectar células cancerosas, que comprende:

(a) exponer células a un compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 o la reivindicación 2; y

(b) determinar la magnitud de unión del compuesto de conjugado anticuerpo-medicamento a las células.

57. El análisis de la reivindicación 56, en donde las células son células tumorales de mama.

58. El análisis de la reivindicación 56, en donde la magnitud de unión se determina midiendo los niveles de ácido nucleico que codifica ErbB por hibridación fluorescente in situ (FISH).

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59. El análisis de la reivindicación 56, en donde la magnitud de unión se determina por inmunohistoquímica (IHC).

60. Un artículo de fabricación que comprende

un compuesto de conjugado anticuerpo-medicamento de la reivindicación 1 ó la reivindicación 2;

un envase; y

un prospecto de envase o etiqueta que indica que el compuesto puede usarse para tratar cáncer caracterizado por la sobreexpresión de un receptor de ErbB.

61. El artículo de fabricación de la reivindicación 60, en donde dicho prospecto de envase o etiqueta indica que el compuesto puede usarse para tratar cáncer caracterizado por la sobreexpresión de un receptor ErbB2.

62. El artículo de fabricación de la reivindicación 61, en donde el cáncer es cáncer de mama.

63. El artículo de fabricación de la reivindicación 62, en donde el cáncer se caracteriza por la sobreexpresión de un receptor ErbB2 en un nivel 2+ o superior.

64. Un método de elaborar un compuesto de conjugado anticuerpo-medicamento, que comprende un anticuerpo, unido covalentemente por un enlazador, a una o más fracciones de medicamento maitansinoide, compuesto que tiene la Fórmula I:

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

o su sal farmacéuticamente aceptable o solvato, en donde:

Ab es un anticuerpo que se une a un receptor ErbB, o que se une a uno o más antígenos asociados a un tumor o receptores de superficie celular seleccionados de (1)-(36):

(1) BMPR1B (proteína morfogenética ósea tipo receptor IB, No. Acceso a Genbank NM_001203);

(2) E16 (LAT1, SLC7A5, No. Acceso a Genbank NM_003486);

(3) STEAP1 (antígeno epitelial de transmembrana de seis hélices de próstata, No. Acceso a Genbank NM_012449);

(4) 0772P (CA125, MUC16, No. Acceso a Genbank AF361486);

(5) MPF (MPF, MSLN, SMR, factor potenciador de megacariocito, mesotelina, No. Acceso a Genbank NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, familia portadora de soluto 34 (fosfato de sodio), miembro 2, transportador 3b de fosfato dependiente de sodio tipo II, No. Acceso a Genbank NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaforina 5b Hlog, dominio sema, siete repeticiones de trombospondina (tipo 1 y similar a tipo 1), dominio de transmembrana (TM) y dominio citoplásmico corto, (semaforina) 5B, No. Acceso a Genbank AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, No. Acceso a Genbank AY358628);

(9) ETBR (receptor tipo B de endotelina, No. Acceso a Genbank AY275463);

(10) MSG783 (RNF124, proteína hipotética FLJ20315, No. Acceso a Genbank NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, gen 1 asociado con cáncer de próstata, proteína 1 asociada con cáncer de próstata, antígeno epitelial de trasmembrana de seis hélices de próstata 2, proteína de próstata de transmembrana de seis hélices, No. Acceso a Genbank AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, canal de catión potencial de receptor transitorio, subfamilia M, miembro 4, No. Acceso a Genbank NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, factor de crecimiento derivado de teratocarcinoma, No. Acceso a Genbank NP_003203 ó NM_003212);

(14) CD21 (CR2 (receptor 2 de Complemento) o C3DR (receptor del virus C3d/Epstein Barr) o Hs.73792 No. Acceso a Genbank M26004);

(15) CD79b (CD79B, CD79β, IGb (beta asociada a inmunoglobulina), B29, No. Acceso a Genbank NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (dominio SH2 que contiene proteína 1a de anclaje fosfatasa), SPAP1B, SPAP1C, No. Acceso a Genbank NM_030764);

(17) HER2 (No. Acceso a Genbank M11730);

(18) NCA (No. Acceso a Genbank M18728);

(19) MDP (No. Acceso a Genbank BC017023);

(20) IL20Rα (No. Acceso a Genbank AF184971);

(21) Brevican (No. Acceso a Genbank AF229053);

(22) EphB2R (No. Acceso a Genbank NM_004442);

(23) ASLG659 (No. Acceso a Genbank AX092328);

(24) PSCA (No. Acceso a Genbank AJ297436);

(25) GEDA (No. Acceso a Genbank AY260763;

(26) BAFF-R (receptor de factor activador de linfocito B, receptor BLyS 3, BR3, NP_443177.1);

(27) CD22 (isoforma CD22-B de receptor de linfocito B, NP-001762.1);

(28) CD79a (CD79A, CD79α, alfa asociada a inmunoglobulina, una proteína específica de linfocito B que interactúa covalentemente con Ig beta (CD79B) y forma un complejo en la superficie con moléculas IgM, transduce una señal involucrada en la diferenciación de linfocitos B, No. Acceso a Genbank NP_001774.1);

(29) CXCR5 (receptor 1 de linfoma 1 de Burkitt, un receptor acoplado a proteína G, que se activa por la quimiocina CXCL13, funciona en migración de linfocitos y defensa humoral, juega un papel en la infección HIV-2 y, quizá, desarrollo de SIDA, linfoma, mieloma, y leucemia, No. Acceso a Genbank NP_001707.1);

(30) HLA-DOB (subunidad Beta de la molécula clase II MHC (antígeno Ia), que une péptidos y los presenta a linfocitos T CD4+, No. Acceso a Genbank NP_002111.1);

(31) P2X5 (canal iónico 5, abierto por acción de ligando P2X, de receptor purinérgico, un canal iónico abierto por ATP extracelular, puede estar involucrado en la transmisión sináptica y neurogénesis; la deficiencia puede contribuir a la patofisiología de inestabilidad detrusora idiopática, No. Acceso a Genbank NP_002552.2);

(32) CD72 (antígeno CD72 de diferenciación de linfocitos B, Lyb-2, No. Acceso a Genbank NP_001773.1);

(33) LY64 (antígeno 64 de linfocitos (RP105), proteína de membrana tipo I de la familia de repeticiones rica en leucina (LRR), regula la activación y apoptosis de linfocitos B; la pérdida de función se asocia con actividad patológica incrementada en pacientes con lupus sistémico eritematoso, No. Acceso a Genbank NP_005573.1);

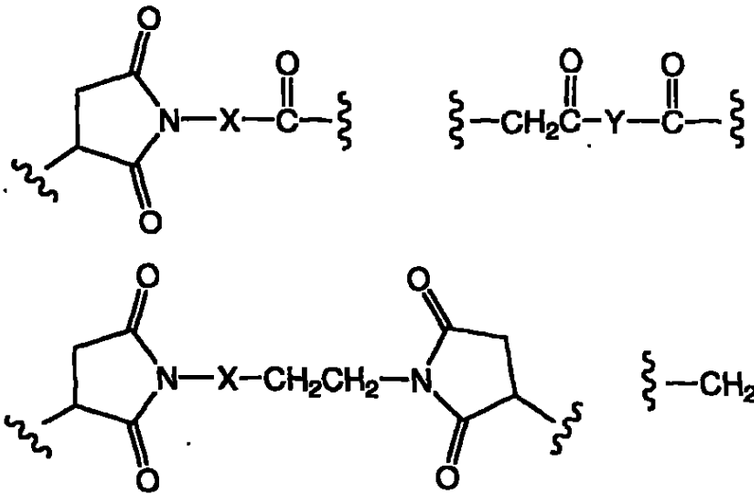
(34) FcRH1 (proteína 1 similar a receptor Fc, un receptor putativo para el dominio Fc de inmunoglobulina que contiene dominios similares a Ig tipo C2 y dominios ITAM, puede tener un papel en la diferenciación de linfocitos B, No. Acceso a Genbank NP_443170.1);

(35) IRTA2 (asociado 2 de translocación de receptor de superfamilia de inmunoglobulina, un inmunorreceptor putativo con posibles papeles en el desarrollo de linfocitos B y génesis de linfoma; la desregulación del gen por translocación ocurre en algunas neoplasias maligna de linfocitos B, No. Acceso a Genbank NP_112571.1); y

(36) TENB2 (proteoglucano de transmembrana putativo, relacionado con la familia EGF/herregulina de factores de crecimiento y folistatina, No. Acceso a Genbank AF179274;

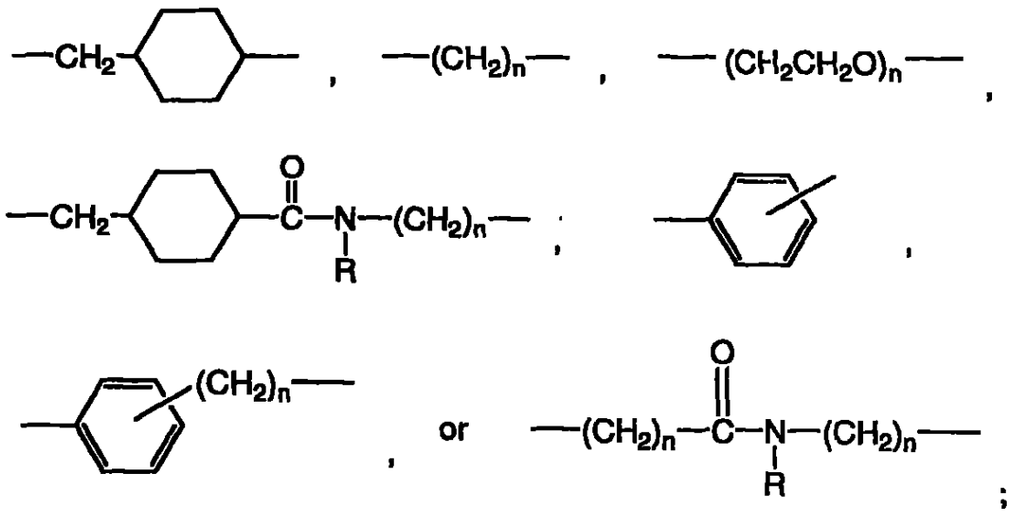
siempre que el anticuerpo no sea TA.1.

L es un enlazador seleccionado de las estructuras:

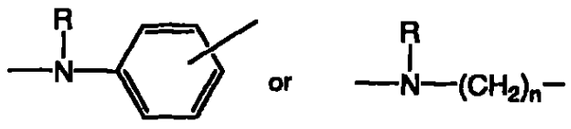


donde las líneas onduladas indican las uniones covalentes a Ab y D;

X es:



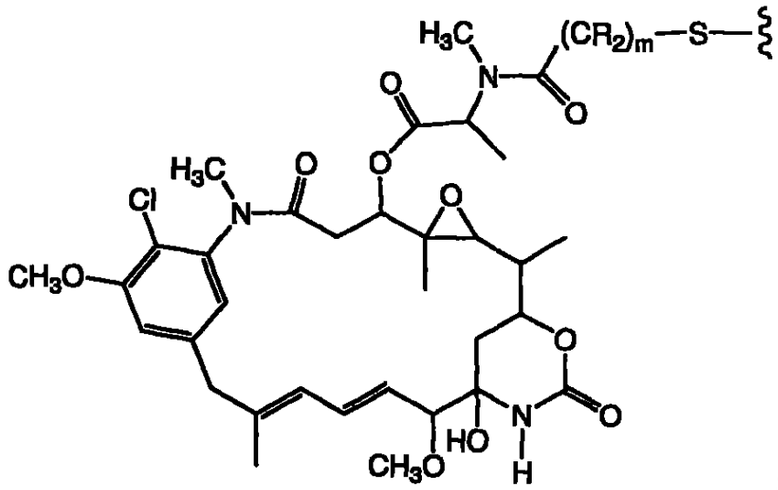
Y es:



en donde R es independientemente H ó C_1-C_6 alquilo; y n es 1 a 12;

D es una fracción de medicamento maitansinoide seleccionada de la estructura:

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donde la línea ondulada indica la unión covalente a L; R es independientemente H ó C_1-C_6 alquilo; m es 1, 2, ó 3; y p es 1 a 8;

en donde el método comprende:

hacer reaccionar Ab con un reactivo enlazador para formar el intermediario anticuerpo-enlazador Ab-L y, luego, hacer reaccionar Ab-L con una fracción de medicamento D para formar el conjugado anticuerpo-medicamento; o

hacer reaccionar una fracción de medicamento D con un reactivo enlazador para formar un intermedio medicamento-enlazador D-L y, luego, hacer reaccionar D-L con Ab para formar el conjugado anticuerpo-medicamento.

65. El método de la reivindicación 64, en donde Ab es un anticuerpo que se une a uno o más antígenos asociados con tumores o receptores de superficie celular seleccionados de (1)-(16) y (18)-(36).

66. El método de la reivindicación 64 ó la reivindicación 65, en donde el reactivo enlazador es SMCC.

67. El método de la reivindicación 64 ó la reivindicación 65, donde el reactivo enlazador es un reactivo de bis-maleimida seleccionado de DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, y $BM(PEO)_4$.

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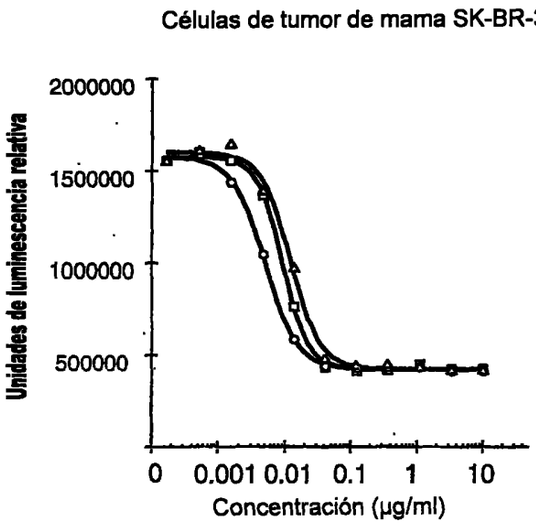


Figura 1

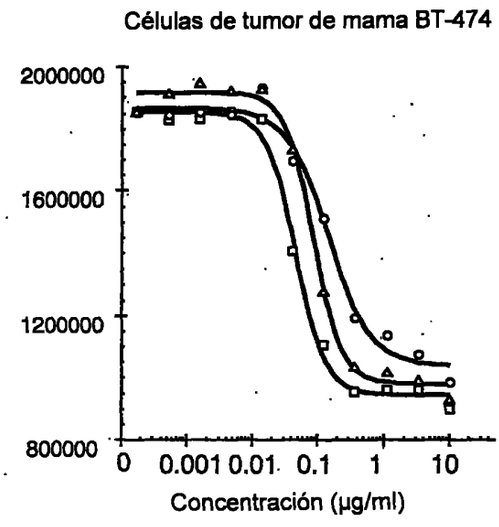


Figura 2

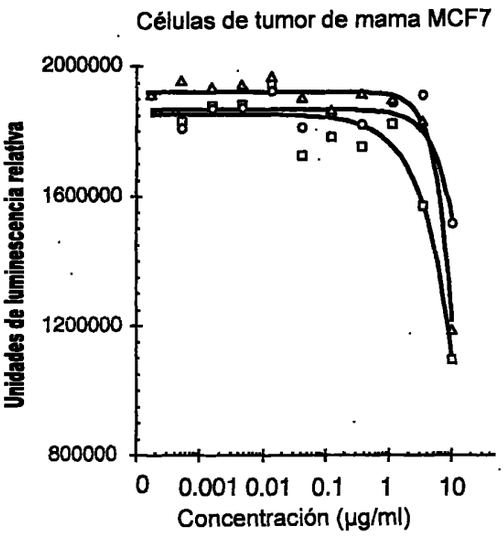


Figura 3

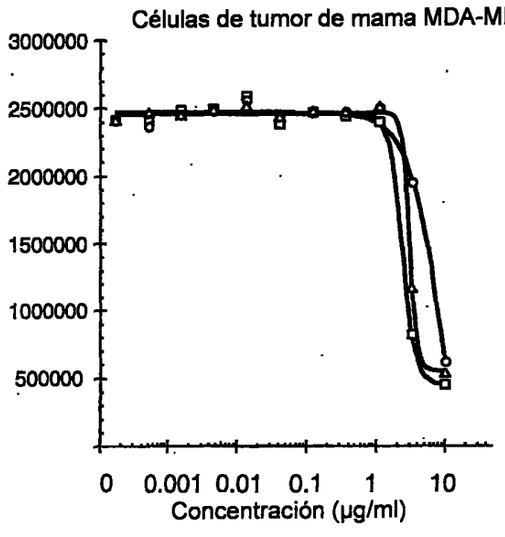


Figura 4

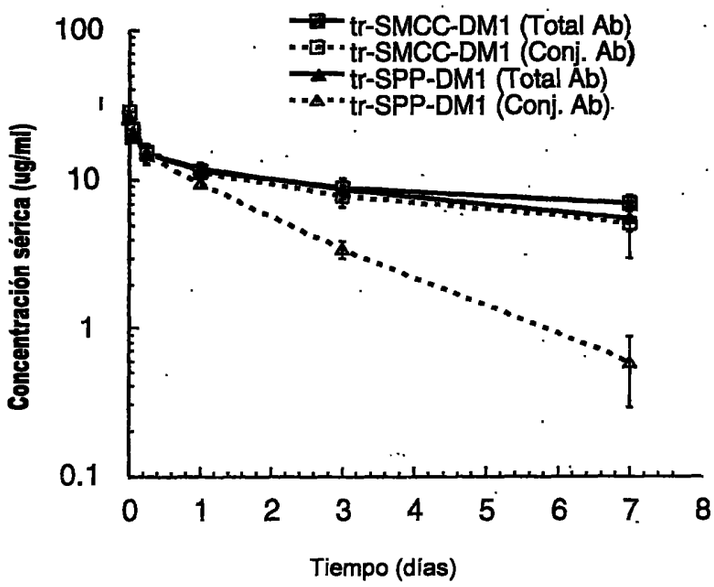

tr-SMCC-DM1 (Total Ab)
tr-SMCC-DM1 (Conj. Ab)
tr-SPP-DM1 (Total Ab)
tr-SPP-DM1 (Conj. Ab)
Concentración sérica (ug/ml)
100
10
1
0.1
0
1
2
3
4
5
6
7
8
Tiempo (días)

Figura 5

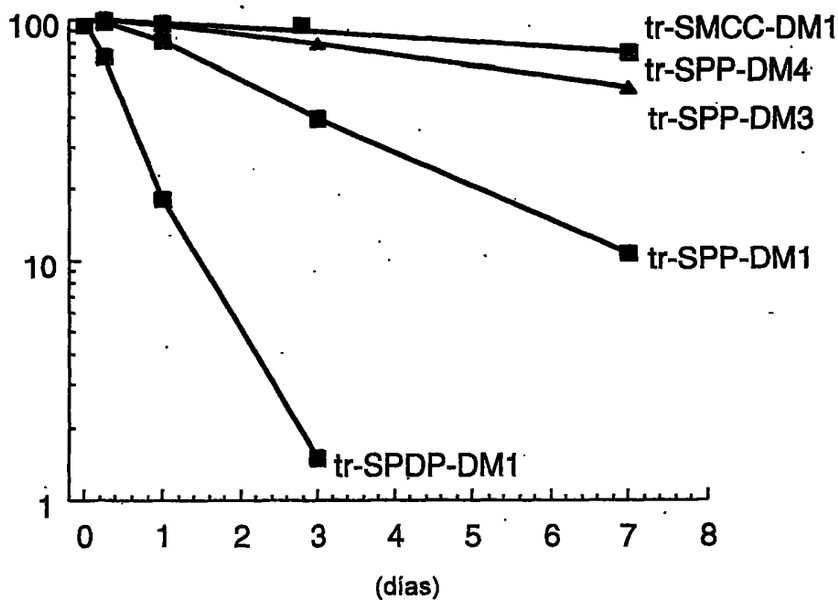

100
10
1
0
1
2
3
4
5
6
7
8
(días)
tr-SMCC-DM1
tr-SPP-DM4
tr-SPP-DM3
tr-SPP-DM1
tr-SPDP-DM1

Figura 6

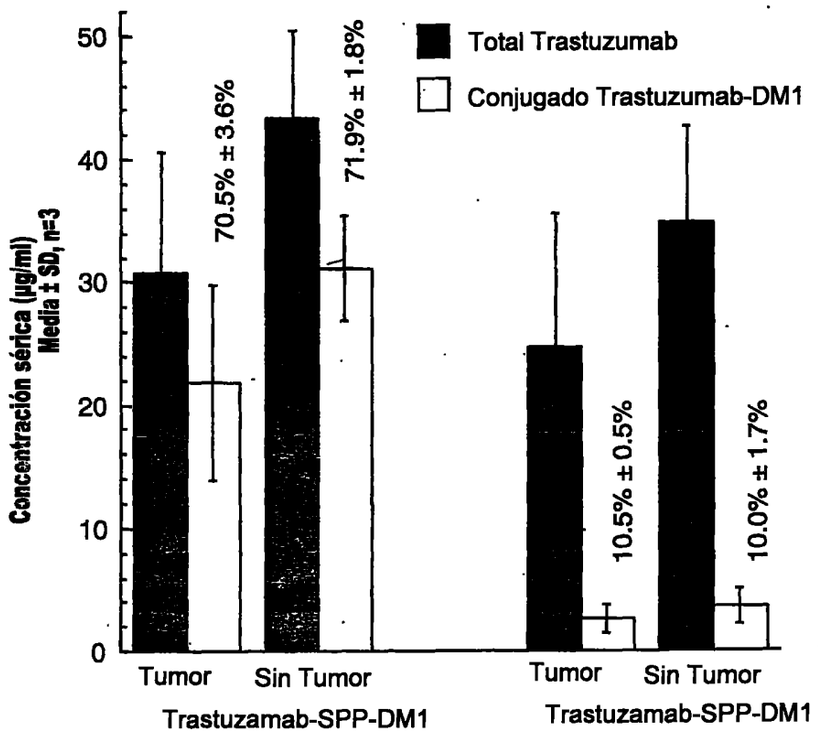

Concentración sérica (µg/ml)
Media ± SD, n=3
50
40
30
20
10
0
Total Trastuzumab
Conjugado Trastuzumab-DM1
70.5% ± 3.6%
71.9% ± 1.8%
10.5% ± 0.5%
10.0% ± 1.7%
Tumor
Sin Tumor
Trastuzamab-SPP-DM1
Tumor
Sin Tumor
Trastuzamab-SPP-DM1

Figura 7

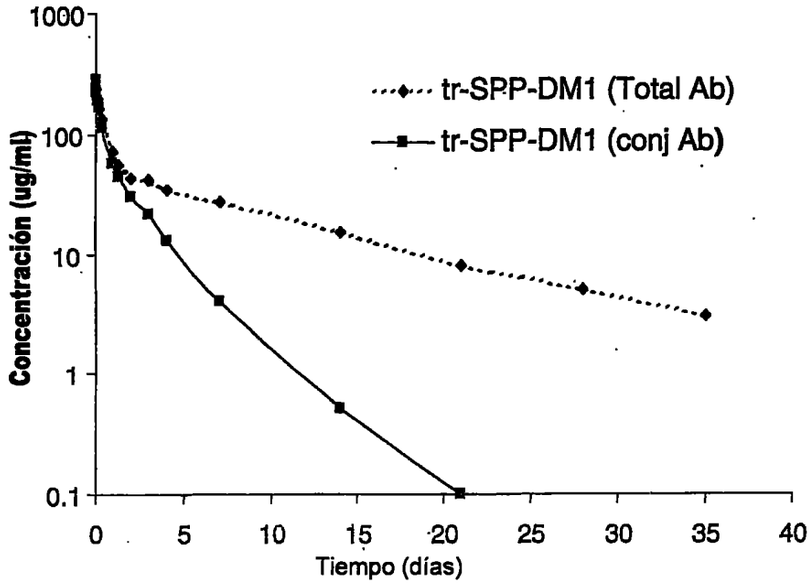

1000
100
10
1
0.1
Concentración (ug/ml)
0
5
10
15
20
25
30
35
40
Tiempo (días)
tr-SPP-DM1 (Total Ab)
tr-SPP-DM1 (conj Ab)

Figura 8

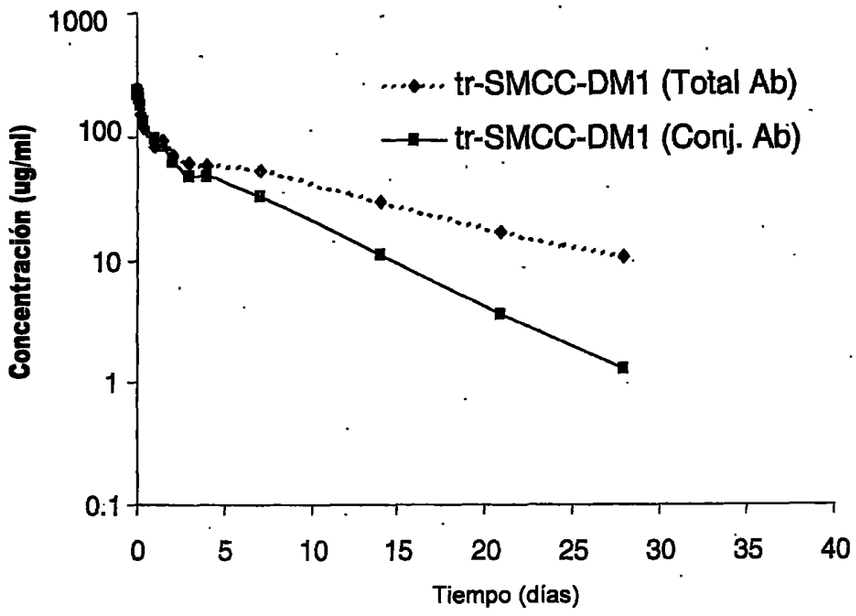

1000
100
10
1
0.1
Concentración (ug/ml)
0
5
10
15
20
25
30
35
40
Tiempo (días)
tr-SMCC-DM1 (Total Ab)
tr-SMCC-DM1 (Conj. Ab)

Figura 9

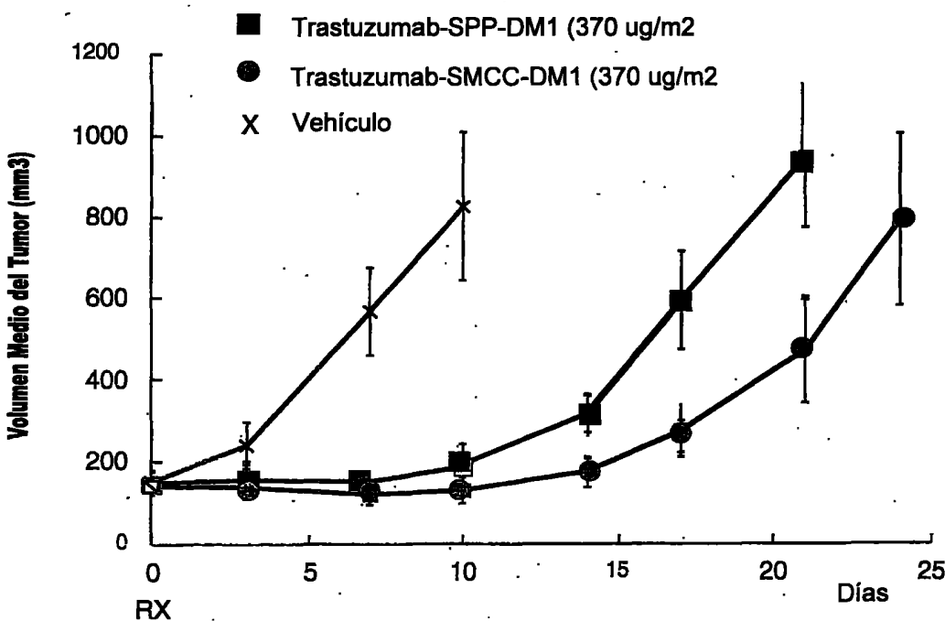

Trastuzumab-SPP-DM1 (370 ug/m2
Trastuzumab-SMCC-DM1 (370 ug/m2
X Vehículo
Volumen Medio del Tumor (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
RX
Días

Figura 10

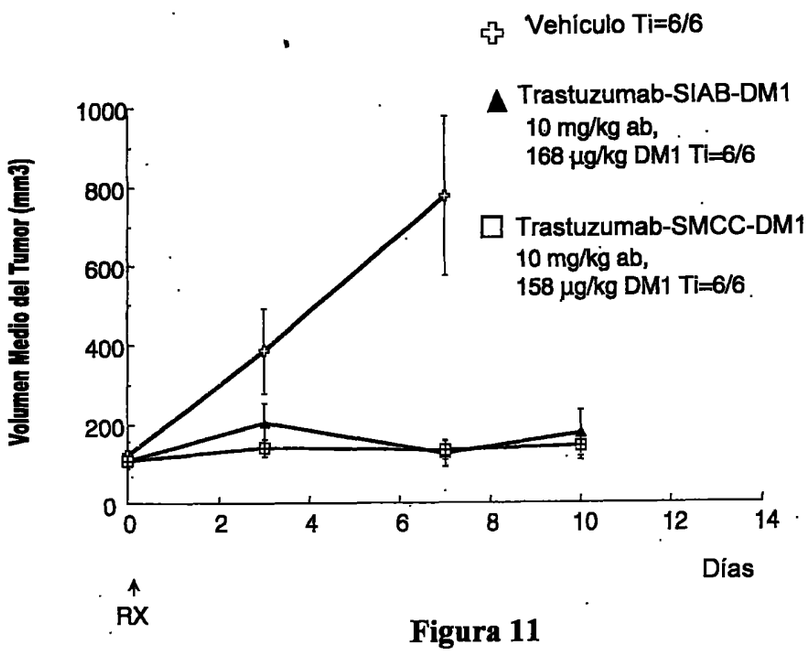

Vehículo Ti=6/6
Trastuzumab-SIAB-DM1
10 mg/kg ab,
168 µg/kg DM1 Ti=6/6
Trastuzumab-SMCC-DM1
10 mg/kg ab,
158 µg/kg DM1 Ti=6/6
Volumen Medio del Tumor (mm3)
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
Días
RX

Figura 11

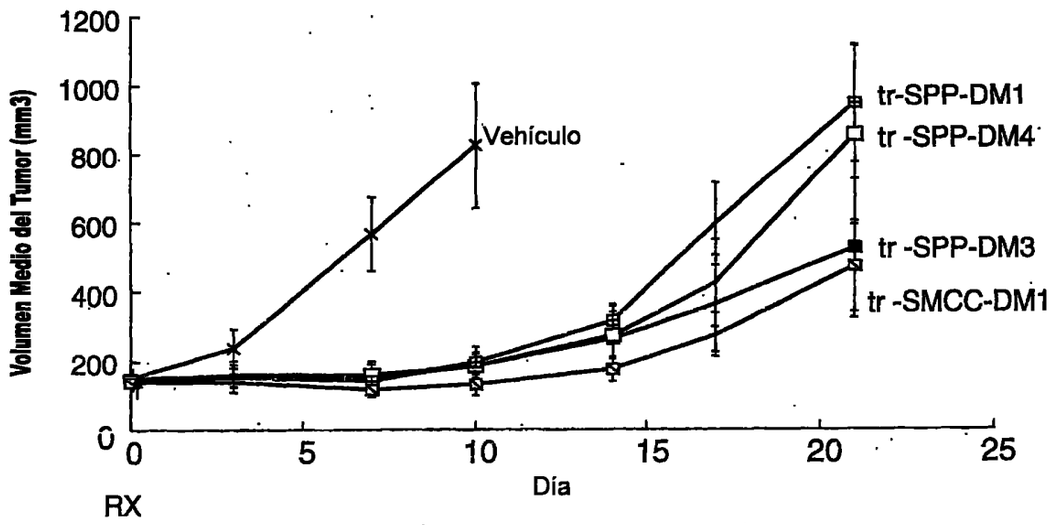
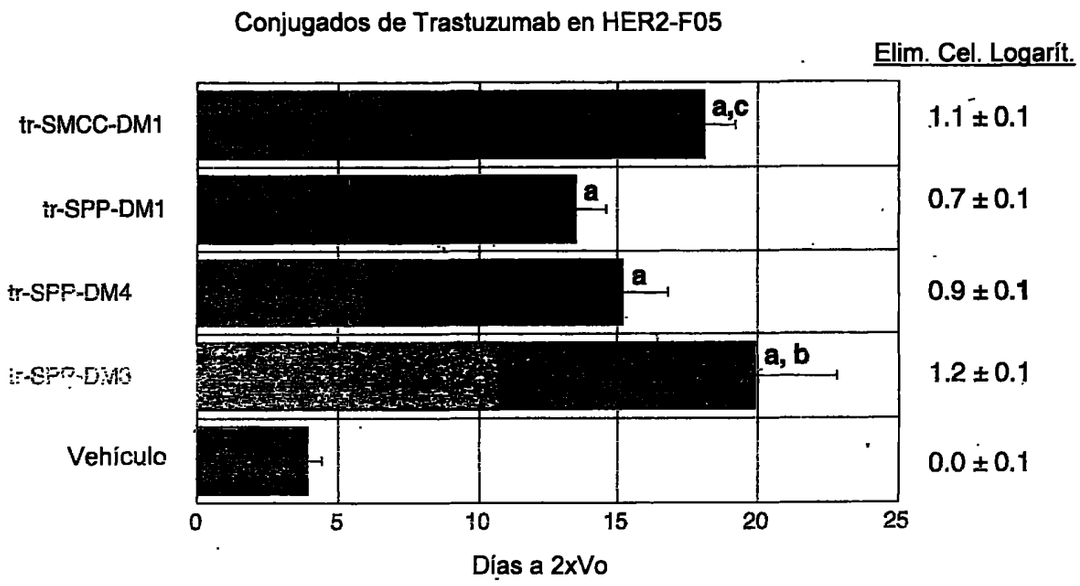

Volumen Medio del Tumor (mm3)
1200
1000
800
600
400
200
0
0
5
10
15
20
25
Día
RX
Vehículo
tr-SPP-DM1
tr -SPP-DM4
tr -SPP-DM3
tr -SMCC-DM1

Figura 12



a P<0.0003 vs vehículo b P=0.010 vs tr-SPP-DM1 c P=0.61 vs tr-SPP-DM1

Figura 13

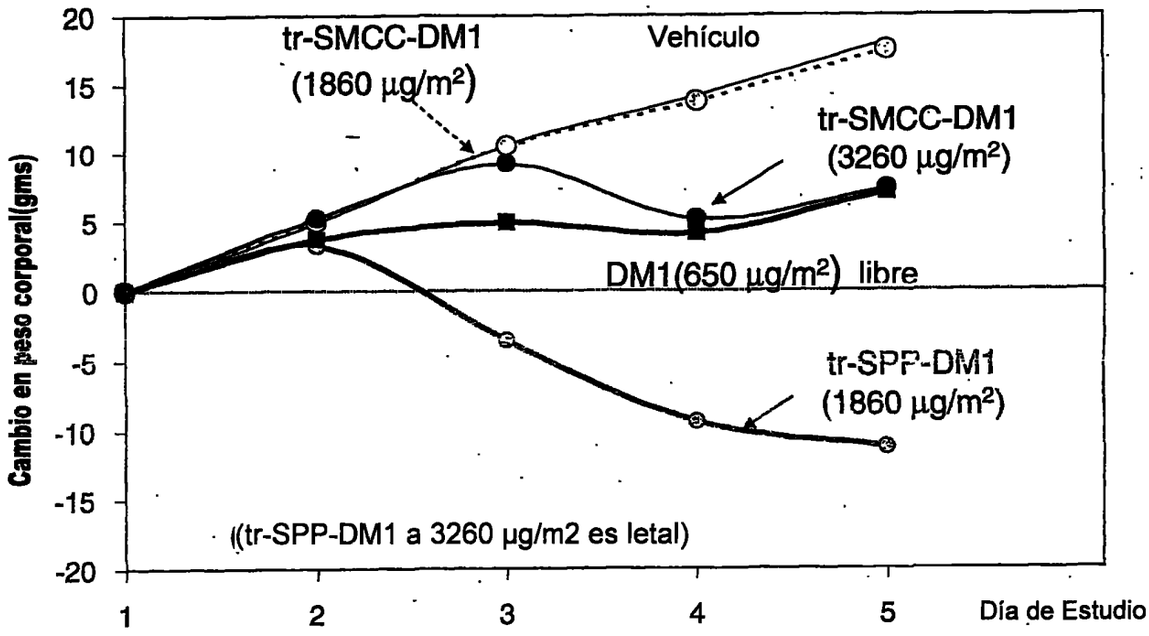
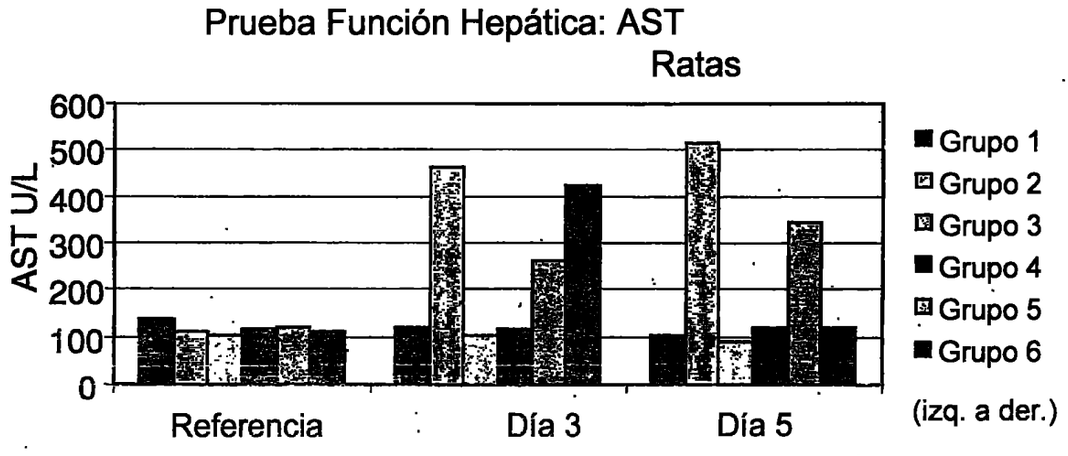

tr-SMCC-DM1
(1860 µg/m²)
Vehículo
tr-SMCC-DM1
(3260 µg/m²)
DM1(650 µg/m²) libre
tr-SPP-DM1
(1860 µg/m²)
((tr-SPP-DM1 a 3260 µg/m2 es letal)
Cambio en peso corporal(gms)
Día de Estudio
20
15
10
5
0
-5
-10
-15
-20
1
2
3
4
5

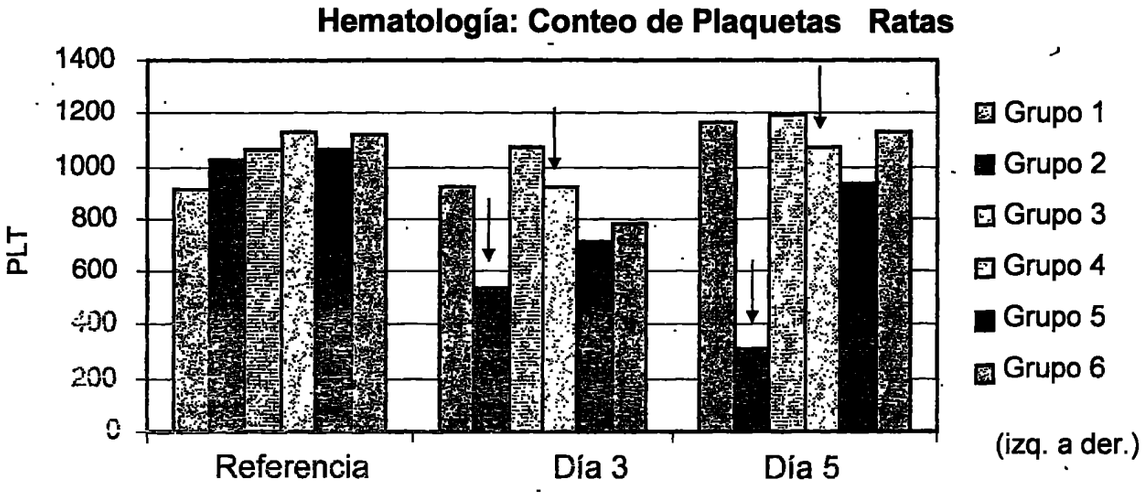
Figura 14



Grupo 1: Vehículo
Grupo 2: tr-SPP-DM1, 22.3mg/kg
Grupo 3: tr-SMCC-DM1, 10mg/kg
Grupo 4: tr-SMCC-DM1, 25mg/kg
Grupo 5: tr-SMCC-DM1, 50mg/kg
Grupo 6: DM1 libre

Figura 15

WO 2005/117986 PCT/US2005/018829



Grupo 1: vehículo
Grupo 2: tr-SPP-DM1, 22.3mg/kg
Grupo 3: tr-SMCC-DM1, 10mg/kg
Grupo 4: tr-SMCC-DM1, 25mg/kg
Grupo 5: tr-SMCC-DM1, 50mg/kg
Grupo 6, free DM1

Figura 16

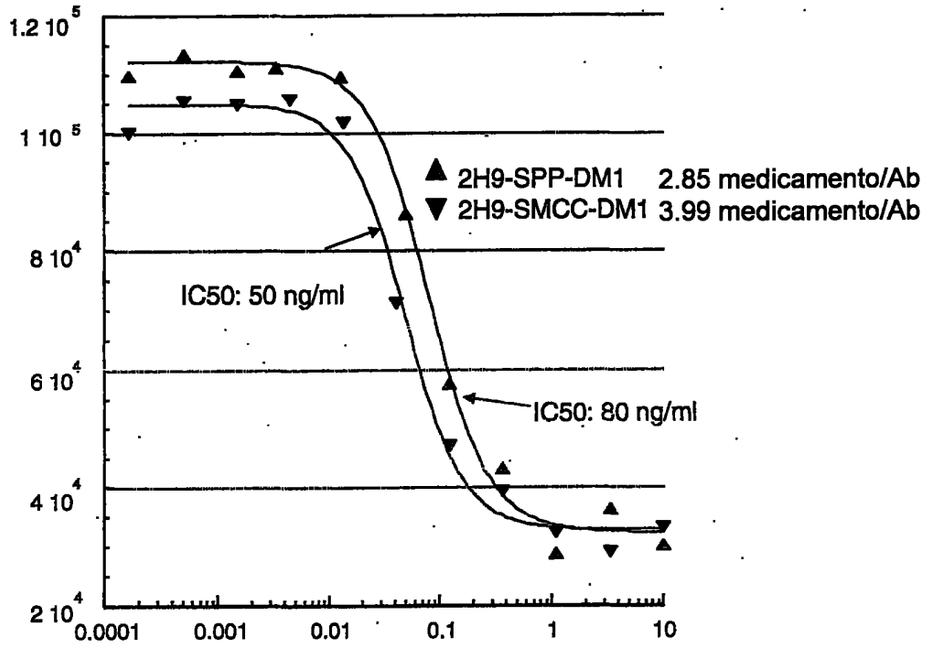

1.2 10^5
1 10^5
8 10^4
6 10^4
4 10^4
2 10^4
0.0001
0.001
0.01
0.1
1
10
2H9-SPP-DM1 2.85 medicamento/Ab
2H9-SMCC-DM1 3.99 medicamento/Ab
IC50: 50 ng/ml
IC50: 80 ng/ml

Figura 17

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PATENT COOPERATION TREAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
GENENTECH, INC.
Attn. Andrus, Alex
1 DNA Way MS49
South San Francisco, CA 94080
UNITED STATES OF AMERICA

6 Apr 2006

RECEIVED
APR 06 2006
GENENTECH, INC.
LEGAL DEPT.

Applicant's or agent's file reference: P2094R1	Date of mailing (day/month/year): 03/04/2006
International application No.: PCT/US2005/018829	FOR FURTHER ACTION: See paragraphs 1 and 4 below
Applicant: GENENTECH, INC.	International filing date (day/month/year): 31/05/2005

Calendared/CLK
Consider Resp/Ch 2
8 JUN 06
Chapter II - 3 JUL 06
30 DAY US ISA - P2094R1 - 3 MAY 06

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

   Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority	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	Stefanie Büchler

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the International application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 48.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more *new* claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1*bis*(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion 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 (Rule 43*bis*.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.

PATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
P2094R1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2005/018829	31/05/2005	01/06/2004
Applicant		
GENENTECH, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 11 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report
   a. With regard to the language, the international search was carried out on the basis of:
      [X] 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))
   b. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. [X] Certain claims were found unsearchable (See Box No. II)

3. [X] Unity of invention is lacking (see Box No III)

4. With regard to the title,
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. 10
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [X] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/018829

Box 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 35-55 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 dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box 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:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-10, 13, 15-67 (partially) and 11 (completely)

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.

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1, 3-8, 10, 15-64, 66 (partially) and 11(completely)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the first molecule mentionned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -CH2-cyclohexane-

---

Invention 2: claims 1, 3-7, 15-64 (partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the first molecule mentionned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -(CH2)n-

---

Invention 3: claims 1, 3-7, 15-64 (partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the first molecule mentionned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -(CH2-CH2-O)n-

---

Invention 4: claims 1, 3-7, 15-64 (partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the first molecule mentionned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -CH2-cyclohexane-CO-NR-(CH2)n- or -(CH2)n-CO-NR-(CH2)n-

---

Invention 5: claims 1, 3-7, 15-64 (partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the first molecule mentionned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -benzene- or -benzene-(CH2)n-

---

Invention 6: claims 1, 3-6, 9, 15-64 (partially) and 12 (completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the second molecule mentionned in claim 3 (second molecule of the first line of molecules of claim 3 (-CH2-CO-Y-CO-))

Invention 7: claims 1, 3-6, 13, 15-64, 67 (partially) and 14 (completely)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the third molecule mentionned in claim 3 (first molecule of the second line of molecules of claim 3 (-maleimide-X-CH2-CH2-maleimide-))

Invention 8: claims 1, 3-6, 15-64 (partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker as the structure of the fourth molecule mentionned in claim 3 (second molecule of the second line of molecules of claim 3 (-CH2-CO-))

Invention 9-43: claims 1-10, 13, 15, 20, 23-30, 33-67 (all partially)

Subject-mater related to an antibody-drug conjugate wherein the antibody binds to one of the molecules (1)-(16), (18)-(36) according to claim 1, each of the binding molecules defines one independant invention (the molecule (17) is not included since Her2 is an erbB receptor and therefore belongs to the inventions 1-8).

International application No
PCT/US2005/018829

A. CLASSIFICATION OF SUBJECT MATTER
A61K47/48 A61P35/00 G01N33/574

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)
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, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/00244 A (GENENTECH, INC; ERICKSON, SHARON; SCHWALL, RALPH; SLIWKOWSKI, MARK, X) 4 January 2001 (2001-01-04) page 37, line 5 - line 30; claims 17,18,25,28,29,31,32,37-41; figure 1 page 10, line 10 - line 13 page 14, line 11 - line 27 page 38, line 13 - line 19 page 38, line 35 - page 43, line 3 -/-	1,3-8, 10,11, 15-64,66

[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
19 January 2006	03. 04. 2006

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	Loubradou, G

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

International application No
PCT/US2005/018829

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHARI R V J ET AL: "IMMUNOCONJUGATES CONTAINING NOVEL MAYTANSINOIDS: PROMISING ANTICANCER DRUGS" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 52, no. 1, January 1992 (1992-01), pages 127-131, XP000453560 ISSN: 0008-5472 page 127, right-hand column, paragraph 3; figure 2 page 128, right-hand column, last paragraph - page 129, left-hand column, paragraph 3	1-8,10, 15,20, 23,25, 64-66
Y	EP 0 425 235 A (IMMUNOGEN INC) 2 May 1991 (1991-05-02) page 10, line 52 - line 55 page 9, line 5 - line 42; examples 3,4	1-8,10, 15,20, 23,25, 64-66
Y	WO 01/24763 A (IMMUNOGEN, INC; CHARI, RAVI, V., J) 12 April 2001 (2001-04-12) page 21, line 13 - page 22, line 2 page 17, line 3 - page 19, line 3	1-8,10, 15,20, 23,25, 64-66
A	TRAIL P A ET AL: "EFFECT OF LINKER VARIATION ON THE STABILITY, POTENCY, AND EFFICACY OF CARCINOMAREACTIVE BR64-DOXORUBICIN IMMUNOCONJUGATES" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 57, 1 January 1997 (1997-01-01), pages 100-105, XP001019049 ISSN: 0008-5472 the whole document	
P,X	WO 2005/037992 A (IMMUNOGEN, INC; STEEVES, RITA; LUTZ, ROBERT; CHARI, RAVI; XIE, HONGSHE) 28 April 2005 (2005-04-28) cited in the application page 12, paragraph '64!, page 20, paragraph '94!, page 23 paragraph '112! to page 25 paragraph '116! and page 30 paragraph '141! to page 31 paragraph '143! figures 3,4,6a-33,35,36; examples 1a-4	1,3-8, 10,11, 15-64,66

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International application No
PCT/US2005/018829

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 2004/016225 A (GENENTECH, INC) 26 February 2004 (2004-02-26) cited in the application page 82, line 5 - line 35; figures 96,140 page 104, line 3 - page 109, line 26	1-10,13, 15,20, 23-30, 33-49, 56-67
X	WO 03/000113 A (GENENTECH, INC) 3 January 2003 (2003-01-03) page 103, line 19 - page 109, line 6 page 81, line 31 - page 83, line 2 page 5, line 23 - line 26; figures 51,129	1-10,13, 15,20, 23-30, 33-49, 56-67
X	WO 03/024392 A (GENENTECH, INC) 27 March 2003 (2003-03-27) page 100, line 31 - page 106, line 11 page 79, line 29 - page 80, line 21; figure 97	1-10,13, 15,20, 23-30, 33-49, 56-67
X	WO 02/16429 A (GENENTECH, INC; GODDARD, AUDREY; GODOWSKI, PAUL, J; GURNEY, AUSTIN, L;) 28 February 2002 (2002-02-28) page 5, line 27 - line 30; figure 9 page 52, line 24 - page 53, line 32 page 71, line 33 - page 77, line 3	1-10,13, 15,20, 23-30, 33-49, 56-67
Y	WO 03/022995 A (AGENSYS, INC) 20 March 2003 (2003-03-20) page 35, paragraph 5 - page 37, paragraph 4 page 50, paragraph 2 - page 51, paragraph 4	1-10,13, 15,20, 23-30, 33-49, 56-67
X Y	WO 2004/005470 A (IMMUNOGEN, INC) 15 January 2004 (2004-01-15) abstract; example 14 page 5, paragraph 3 page 25, paragraph 2 - paragraph 3	1,2 3-10,13, 15,20, 23-30, 33-49, 56-67

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HU XIU FENG ET AL: "Discovery and validation of new molecular targets for ovarian cancer." CURRENT OPINION IN MOLECULAR THERAPEUTICS. DEC 2003, vol. 5, no. 6, December 2003 (2003-12), pages 625-630, XP009059821 ISSN: 1464-8431 page 627, right-hand column, paragraph 2 - page 628, left-hand column, paragraph 3	1-10,13, 15,20, 23-30, 33-49, 56-67
A	CHU P G ET AL: "CD79: a review." APPLIED IMMUNOHISTOCHEMISTRY & MOLECULAR MORPHOLOGY : AIMM / OFFICIAL PUBLICATION OF THE SOCIETY FOR APPLIED IMMUNOHISTOCHEMISTRY. JUN 2001, vol. 9, no. 2, June 2001 (2001-06), pages 97-106, XP009059897 ISSN: 1541-2016 the whole document	
Y	WO 03/072036 A (DUKE UNIVERSITY; UNIVERSITY OF CALIFORNIA; TEDDER, THOMAS; TUSCANO, JO) 4 September 2003 (2003-09-04) page 18, paragraph 5 - page 19, paragraph 1 page 19, paragraph 3 - page 22, last paragraph	1-10,13, 15,20, 23-30, 33-49, 56-67
Y	WO 00/74718 A (IMMUNOMEDICS, INC; GOLDENBERG, DAVID, M; HANSEN, HANS, J) 14 December 2000 (2000-12-14) page 16, line 19 - page 17, line 14 page 8, line 17 - page 10, line 17 abstract	1-10,13, 15,20, 23-30, 33-49, 56-67
A	WO 03/027135 A (THE GOVERNMENT OF THE UNITED STATES, AS REPRESENTED BY THE SECRETARY O) 3 April 2003 (2003-04-03) abstract page 3, line 16 - page 4, line 7 page 31, line 20 - page 32, line 14	1-10,13, 15,20, 23-30, 33-49, 56-67

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/38490 A (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 31 May 2001 (2001-05-31) cited in the application abstract page 9, line 25 - line 30 page 45, line 21 - line 27	1-10,13, 15,20, 23-30, 33-49, 56-67
Y	WO 03/075855 A (EOS BIOTECHNOLOGY, INC; BHASKAR, VINAY; DE LA CALLE, AGUSTIN; LAW, DEB) 18 September 2003 (2003-09-18) page 27, last paragraph - page 28, paragraph 1 page 4, paragraph 3 page 3, paragraph 1 abstract	1-10,13, 15,20, 23-30, 33-49, 56-67
Y	US 5 416 064 A (CHARI ET AL) 16 May 1995 (1995-05-16) column 12, line 27 - line 54	1-10,13, 15,20, 23-30, 33-49, 56-67
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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/018829	31.05.2005	01.06.2004

International Patent Classification (IPC) or both national classification and IPC
A61K47/48, A61P35/00, G01N33/574

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"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified 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 three 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:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/018829

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, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. 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:

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ 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.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/018829

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. claims 1-10, 13, 15-67 (partially) and 11(completely)

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	9, 13,56-59, 67
	No:	Claims	1-8, 10, 11, 15-55, 60-66
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11, 13, 15-67
Industrial applicability (IA)	Yes:	Claims	1-11, 13, 15-34, 56-67
	No:	Claims	

2. Citations and explanations

   see separate sheet

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/018829

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2005/018829

Reference is made to the following documents:

D1: CHARI R V J ET AL: "IMMUNOCONJUGATES CONTAINING NOVEL MAYTANSINOIDS: PROMISING ANTICANCER DRUGS" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, US, vol. 52, no. 1, January 1992 (1992-01), pages 127-131, XP000453560 ISSN: 0008-5472

D2: WO 01/00244 A (GENENTECH, INC; ERICKSON, SHARON; SCHWALL, RALPH; SLIWKOWSKI, MARK, X) 4 January 2001 (2001-01-04)

D3: EP-A-0 425 235 (IMMUNOGEN INC) 2 May 1991 (1991-05-02)

D4: WO 01/24763 A (IMMUNOGEN, INC; CHARI, RAVI, V., J) 12 April 2001 (2001-04-12)

D5: WO 2004/016225 A (GENENTECH, INC) 26 February 2004 (2004-02-26)

D6: WO 03/000113 A (GENENTECH, INC) 3 January 2003 (2003-01-03)

D7: WO 03/072036 A (DUKE UNIVERSITY; UNIVERSITY OF CALIFORNIA; TEDDER, THOMAS; TUSCANO, JO) 4 September 2003 (2003-09-04)

D8: WO 03/024392 A (GENENTECH, INC) 27 March 2003 (2003-03-27)

D9: WO 02/16429 A (GENENTECH, INC; GODDARD, AUDREY; GODOWSKI, PAUL, J; GURNEY, AUSTIN, L;) 28 February 2002 (2002-02-28)

Re Item IV.

The separate inventions/groups of inventions are:

Invention 1: claims 1, 3-8, 10, 15-64, 66 (partially) and 11(completely)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the first molecule mentioned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -CH2-cyclohexane-.

Invention 2: claims 1, 3-7, 15-64 (partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the first molecule mentioned in claim 3 (first molecule of the first line of molecules of claim 3 (-

maleimide-X-CO-)) and wherein X is -(CH2)n-.

Invention 3: claims 1, 3-7, 15-64 (partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the first molecule mentioned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -(CH2-CH2-O)n-.

Invention 4: claims 1, 3-7, 15-64 (partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the first molecule mentioned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -CH2-cyclohexane-CO-NR-(CH2)n- or -(CH2)n-CO-NR-(CH2)n-.

Invention 5: claims 1, 3-7, 15-64 (partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the first molecule mentioned in claim 3 (first molecule of the first line of molecules of claim 3 (-maleimide-X-CO-)) and wherein X is -benzene- or -benzene-(CH2)n-.

Invention 6: claims 1, 3-6, 9, 15-64 (partially) and 12 (completely)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the second molecule mentioned in claim 3 (second molecule of the first line of molecules of claim 3 (-CH2-CO-Y-CO-)).

Invention 7: claims 1, 3-6, 13, 15-64, 67 (partially) and 14 (completely)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the third molecule mentioned in claim 3 (first molecule of the second line of molecules of claim 3 (-maleimide-X-CH2-CH2-maleimide-)).

Invention 8: claims 1, 3-6, 15-64 (partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to an ErbB receptor and wherein the linker has the structure of the fourth molecule mentioned in claim 3 (second molecule of the second line of molecules of claim 3 (-CH2-CO-)).

Inventions 9-43: claims 1-11, 13, 15, 20, 23-30, 33-67 (all partially)

Subject-matter related to an antibody-drug conjugate wherein the antibody binds to one of the molecules (1)-(16), (18)-(36) according to claim 1, each of the binding molecules defines one independant invention (the molecule (17) is not included since Her2 is an ErbB receptor and therefore belongs to the inventions 1-8).

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

According to Rule 13.1 PCT, an international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention referred to in Rule 13.1 shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art (Rule 13.2 PCT).
The inventions 1 to 43 as defined above only share the technical features of being antibody-drug conjugates binding to a tumor-associated antigen or a cell-surface receptor wherein the antibody drug conjugate has the general formula Ab-(L-D)p, L being a non-disulfite linker, D being a maytansinoid drug moiety and p being 1 to 8. However, such antibody-drug conjugates and their use in the field of cancer research/therapy are known in the art:

- D1 discloses a TA.1-maytansinoid conjugate wherein TA.1 is an antibody directed against Her2/neu (a receptor of the ErbB family) and wherein the linker was obtained using SMCC which does not generate a disulfide linker (see D1, page 127, right-hand column, third paragraph, page 128, right hand column, second paragraph to page 129 left-hand column, third paragraph and the figure 2). The conjugate

There is at present no evidence on file that immunoconjugates having a non-disulfide linkers share any specific properties.

The problem to be solved by the present invention may therefore be regarded as the provision of antibody-maytansinoid conjugates directed against CD22 which can be used in the field of cancer research/treatment.

In order to select the linker and starting from D7, the skilled person would consider the documents referred to in D7 with respect to the maytansinoid immunoconjugates, for example D3 (see D7, page 18 paragraph [0088]). D3 discloses several disulfide and non-disulfide linkers (see D3, page 10, lines 24 to 55 and the examples 3 and 4). Among the preferred linkers is the non-disulfide linker SMCC (see D3, page 10, lines 52 to 55). The selection of one of the preferred linkers specifically mentioned in D3 cannot involve an inventive step.
Therefore claim 1 does not involve an inventive step (Article 33(3) PCT).

The objection applies *mutatis mutandis* to the independant claim 64 (Article 33(3) PCT).

Dependent claims 2 to 10, 13, 15, 20, 23 to 25 and 65 to 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D4 and D7 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

5.2 The immunoconjugates of D7 are clearly directed to the treatment of cancer (see D7, page 19 paragraph [0091] to page 22 paragraph [0102]). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field do not involve an inventive step, see documents D2 to D4 and D7, and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Invention 13 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds IRTA2)

6.1 D8 discloses immunoconjugates directed against several antigens including IRTA2 (see D8, figure 97 and page 5 lines 29 to 33). The maytansinoids are the preferred toxins (see D8, page 79 line 14 to page 80 line 21) and linking agents generating thioether linkage are among the two most preferred types of linking agent (see D8, page 80, lines 4 to 6). SMCC is specifically cited (see D8, page 80 lines 7 to 9).

Therefore, D8 anticipates claims 1 to 3, 7, 8 and 64 to 66 (Article 33(2) PCT).

Dependent claims 4 to 6, 9, 10, 13, 15, 20, 23 to 25 and 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D4 and D8 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

6.2 The immunoconjugates of D8 are clearly directed to the treatment of cancer (see D8, page 100 line 31 to page 106 line 11). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field are not novel in view of D8 or do not involve an inventive step, see documents D2 to D4 and D8 and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Invention 14 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds TENB2)

7.1 D9 discloses immunoconjugates directed against several antigens including TENB2 (see D9, figure 9 and page 5 lines 23 to 31). The maytansinoids are the preferred

toxins (see D9, page 52 line 13 to page 53 line 32) and linking agents generating thioether linkage are among the two most preferred types of linking agents (see D9, page 53, lines 15 to 17). SMCC is specifically cited (see D9, page 53 lines 18 to 20).

Therefore, D9 anticipates claims 1 to 3, 7, 8 and 64 to 66 (Article 33(2) PCT).

Dependent claims 4 to 6, 9, 10, 13, 15, 20, 23 to 25 and 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D4 and D9 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

7.2 The immunoconjugates of D9 are clearly directed to the treatment of cancer (see D9, page 71 line 33 to page 77 line 3). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field are not novel in view of D9 or do not involve an inventive step, see documents D2 to D4 and D9 and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Remarks/objections relevant for inventions 1 and 9 to 14

8. Claims 35 to 55 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).

Re Item VI
Certain documents cited

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2005/018829

Certain published documents

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO2005/037992	28.04.2005	12.10.2004	10.10.2003
WO2005/049075	02.06.2005	16.11.2004	17.11.2003
WO2005/101017	27.10.2005	06.04.2005	07.04.2004

Re Item V.

Invention 1 (subject-matter of claims 1, 3-8, 10, 11, 15-64, 66 as far as the antibody binds to an ErbB receptor and the linker has the formula -maleimide-CH2-cyclohexane-CO-)

1.1 D2 discloses anti-ErbB antibody-maytansinoid conjugates obtained by using SMCC as linking agent (see D2, page 37 lines 4 to 30). The corresponding linker has the formula -maleimide-CH2-cyclohexane-CO-.
The antibody huMAb4D5-8 is the preferred antibody (see the claim 18 and the examples pages 43 to 47) and DM1 is the preferred maytansinoid (see the claim 25 and the example 2). Thioether bonds (which are the bonds generated by using SMCC) are the second most preferred type of bonds (see claim 29 and page 37 lines 12 to 16).
Therefore, D2 anticipates claims 1, 3-8, 10, 11, and 15 to 25 (Article 33(2) PCT).

1.2 The methods of claims 64 and 66 are inevitably used to generate the above mentioned anti-ErbB antibody-maytansinoid conjugates, therefore claims 64 and 66 are also not novel (Article 33(2) PCT).

1.3 D2 further discloses pharmaceutical compositions comprising the above mentioned anti-ErbB antibody-maytansinoid immunoconjugates (including pharmaceutical compositions comprising additional chemotherapeutic agents, see D2, page 38, lines 17 to 19), methods to treat cancer using said immunoconjugates (see D2, page 38, line 35 to page 42 line 4) and articles of manufacture comprising said immunoconjugates (see D2, page 42, line 6 to page 43 line 4). Therefore, the independent claims 26, 30, 35, 50 and 60 are not novel (Article 33(2) PCT).
Dependent claims 27 to 29, 31 to 34, 36 to 49, 51 to 55 and 61 to 63 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see documents D2 to D4 and the corresponding passages cited in the search report.

1.4 It is well known in the art that some cancer cells overexpress ErbB receptors (see D2, page 1 line 25 to page 2 line 8), thus the use of antibodies or immunoconjugates

comprises 1 to 6 molecules of linker-maytansinoid (see D1, page 127, right-hand column, third paragraph). It is noted that TA.1 immunoconjugates are disclaimed in the present claim 1, however this is irrelevant to the present reasoning.

- D2 discloses anti-ErbB antibody-maytansinoid conjugates wherein the linking group can be a disulfide group, a thioether group, an acid labile group, a photolabile group, a peptidase labile group or an esterase labile group (see D2, page 37 lines 5 to 30 and page 38 line 35 to page 42 line 4). Among the linking agents specifically mentioned is SMCC (see D2, page 37, lines 17 to 19). The conjugates comprise 1 to 10, preferably 3 to 5 molecules of linker-maytansinoid (see D2, claims 31 and 32).
- D3 discloses antibody-drug conjugates binding to a tumor-associated antigen or a cell-surface receptor, wherein SMCC is used to conjugate the antibody to the maytansinoid drug (see D3, page 9 lines 5 to 42, page 10 lines 52 to 55 and the example 3). The conjugates comprise 1 to 10 molecules of linker-maytansinoid (see D3, page 10 lines 52 to 55).
- D4 discloses antibody-drug conjugates binding to a tumor-associated antigen or a cell-surface receptor, wherein SMCC is used to conjugate the antibody to the maytansinoid drug (see D4, page 21 line 13 to page 22 line 2 and page 17 line 3 to page 19 line 3).The conjugates comprise 1 to 10 molecules of linker-maytansinoid (see D4, page 21 line 28 to page 22 line 2).

The inventions 1 to 8 further share the technical feature of having one of the linkers of claim 3 and the inventions 1 to 5 also share the technical feature of having a linker having the general formula -maleimide-X-CO-. However, both technical features are not novel in view of D1 and D2 which disclose anti-ErbB antibody-maytansinoid conjugates obtained by using SMCC as linker reagent (using SMCC as linker reagent generates a linker with the formula -maleimide-CH2-cyclohexane-CO-) (see D1, page 127, right-hand column, third paragraph, page 128, right hand column, second paragraph to page 129 left-hand column, third paragraph and figure 2 ,and D2 page 37 lines 5 to 30).

In view of the above reasoning the 43 inventions as defined above do not share a "special technical feature" according to Rule 13.2 PCT and therefore lack unity according to Rule 13.1 PCT.

directed against ErbB receptors in order to detect cancer cells is considered obvious. Therefore, claim 56 does not involve an inventive step (Article 33(3) PCT).
The objection applies *mutatis mutandis* to the dependant claims 57 to 59 (Article 33(3) PCT).

Invention 9 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds STEAP1)

2.1 D5 discloses immunoconjugates directed against several antigens including STEAP1 (see D5, figure 96 and page 5 lines 21 to 24). The maytansinoids are the preferred toxins (see page 81 line 16 to page 82 line 35) and linking agents generating thioether linkage are among the two most preferred types of linking agents (see D5, page 82, lines 18 to 20). SMCC is specifically cited (see D5, page 82 lines 22 to 23).

Therefore, D5 anticipates claims 1 to 3, 7, 8 and 64 to 66 (Article 33(2) PCT).

Dependent claims 4 to 6, 9, 10, 13, 15, 20, 23 to 25 and 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D5 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

2.2 The immunoconjugates of D5 are clearly directed to the treatment of cancer (see D5, page 104 line 3 to page 109 line 2). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field are not novel in view of D5 or do not involve an inventive step, see documents D2 to D5 and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Invention 10 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds 0772P (CA125, MUC16))

3.1 D6 discloses immunoconjugates directed against MUC16 (see D6, figure 129 and page 5 lines 23 to 26). The maytansinoids are the preferred toxins (see page 81 line 31 to page 83 line 2) and linking agents generating thioether linkage are among the two most preferred types of linking agents (see D6, page 82, lines 22 to 24). SMCC is specifically cited (see D6, page 82 lines 25 to 27).

Therefore, D6 anticipates claims 1 to 3, 7, 8 and 64 to 66 (Article 33(2) PCT).

Dependent claims 4 to 6, 9, 10, 13, 15, 20, 23 to 25 and 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D4 and D6 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

3.2 The immunoconjugates of D6 are clearly directed to the treatment of cancer (see D6, page 103 line 19 to page 109 line 5). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field are not novel in view of D6 or do not involve an inventive step, see documents D2 to D4 and D6 and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Invention 11 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds CD79b)

4.1 D5 discloses immunoconjugates directed against several antigens including CD79b (see D5, figure 140 and page 5 lines 18 to 26). The maytansinoids are the preferred toxins (see page 81 line 16 to page 82 line 35) and linking agents generating

thioether linkage are among the two most preferred types of linking agents (see D5, page 82, lines 18 to 20). SMCC is specifically cited (see D5, page 82 lines 22 to 23).

Therefore, D5 anticipates claims 1 to 3, 7, 8 and 64 to 66 (Article 33(2) PCT).

Dependent claims 4 to 6, 9, 10, 13, 15, 20, 23 to 25 and 67 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, see documents D2 to D5 and the corresponding passages cited in the search report.
With respect to claims 9, 13 and 67, it can be noted that the only immunoconjugate having a non-disulfide linker tested in the examples of the present application is Trastuzumab-SMCC-DM1. There is at present no evidence on file suggesting that the properties observed with said immunoconjugate can be extrapolated to immunoconjugates comprising a different antibody and a different linker.

4.2 The immunoconjugates of D5 are clearly directed to the treatment of cancer (see D5, page 104 line 3 to page 109 line 2). Therefore, claims 26 to 49 and claims 56 to 59 which relate to obvious embodiments in this field are not novel in view of D5 or do not involve an inventive step, see documents D2 to D5 and the corresponding passages cited in the search report (Article 33(2) (3) PCT).

Invention 12 (subject-matter of claims 1-10, 13, 15, 20, 23-30, 33-49, 55-67 as far as the antibody binds CD22)

5.1 D7 is regarded as being the closest prior art to the subject-matter of claim 1. D7 discloses antibody-maytansinoid conjugates directed against CD22 (see D7, page 18 paragraph [0086]). The nature of the linker is not mentioned but reference is made to US5208020, US5416020 and EP0425235 (D3) (see D7, page 18 paragraph [0086]). The immunoconjugates of D7 are designed for the treatment of lymphomas and leukemia (see D7, page 1 paragraph [0002])

D7 differs from the subject-matter of the present claim 1 in that the linker is a non-disulfide linker.

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OCT 21 2005

GENENTECH, INC.
LEGAL DEPT.

PATENT COOPERATION TREATY

PCT/US2005/018829

WAK/CS

PCT

From the INTERNATIONAL BUREAU

To:

JWAK

ANDRUS, Alex
Genentech, Inc.
MS 49
1 DNA Way
South San Francisco, CA 94080
ETATS-UNIS D'AMERIQUE

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing *(day/month/year)* 12 October 2005 (12.10.2005)	
Applicant's or agent's file reference P2094R1	IMPORTANT NOTIFICATION
International application No. PCT/US2005/018829	International filing date *(day/month/year)* 31 May 2005 (31.05.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 01 June 2004 (01.06.2004)
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
01 June 2004 (01.06.2004)	60/576,517	US	03 October 2005 (03.10.2005)
05 October 2004 (05.10.2004)	60/616,098	US	03 October 2005 (03.10.2005)

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OCT 21 2005

GENENTECH, INC.
LEGAL DEPT.

ATENT COOPERATION TREAT

PCT/US2005/018829

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

ANDRUS, Alex
Genentech, Inc.
MS 49
1 DNA Way
South San Francisco, CA 94080
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 12 October 2005 (12.10.2005)	
Applicant's or agent's file reference P2094R1	IMPORTANT NOTIFICATION
International application No. PCT/US2005/018829	International filing date *(day/month/year)* 31 May 2005 (31.05.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 01 June 2004 (01.06.2004)
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
01 June 2004 (01.06.2004)	60/576,817	US	03 October 2005 (03.10.2005)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer D. CABOT Facsimile No. (41-22) 338.70.80 Telephone No. +41 22 338 8435

Form PCT/IB/304 (January 2004)

CL3B7FH8

P2094R1

PCT (ANNEX - FEE CALCULATION SHEET)

Original (for SUBMISSION)

(This sheet is not part of and does not count as a sheet of the international application)

0	For receiving Office use only			
0-1	International Application No.			
0-2	Date stamp of the receiving Office			
0-4	Form PCT/RO/101 (Annex) PCT Fee Calculation Sheet			
0-4-1	Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.170)		
0-6	Applicant's or agent's file reference	P2094R1		
2	Applicant	GENENTECH, INC.		
12	Calculation of prescribed fees	fee amount/multiplier	Total amounts (USD)	
12-1	Transmittal fee T	⇨	300	
12-2-1	Search fee S	⇨	2075	
12-2-2	International search to be carried out by	EP		
12-3	International filing fee (first 30 sheets) fi	1211		
12-4	Remaining sheets	85		
12-5	Additional amount (x)	13		
12-6	Total additional amount f2	1105		
12-7	fi + f2 = I	2316		
12-12	EASY Filing reduction R	-87		
12-13	Total international filing fee (I-R) I	⇨	2229	
12-14	Fee for priority document Number of priority documents requested	2		
12-15	Fee per document (X)	20		
12-16	Total priority document fee: P	⇨	40	
12-17	TOTAL FEES PAYABLE (T+S+I+P)	⇨	4644	
12-19	Mode of payment	authorization to charge deposit account		
12-20	Deposit account instructions The receiving Office	United States Patent and Trademark Office (USPTO) (RO/US)		
12-20-1	Authorization to charge the total fees indicated above	✓		
12-20-2	Authorization to charge any deficiency or credit any overpayment in the total fees indicated above	✓		
12-20-3	Authorization to charge the fee for priority document	✓		
12-21	Deposit account No.	07-0630		
12-22	Date	31 May 2005 (31.05.2005)		
12-23	Name and signature	ANDRUS, Alex		

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P2094R1

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 3.50 (Build 0002.170)
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	P2094R1
I	Title of invention	ANTIBODY DRUG CONJUGATES AND METHODS
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 94080-4990 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	1-650-225-1000
II-9	Facsimile No.	1-650-952-9881
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 Ave San Carlos, California 94070 United States of America
III-1-6	State of nationality	US
III-1-7	State of residence	US

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)	JACOBSON, Frederic S.
III-2-5	Address	830 Shattuck Avenue Berkeley, California 94707 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)	POLAKIS, Paul
III-3-5	Address	1449 Cortez Avenue Burlingame, California 94010 United States of America
III-3-6	State of nationality	US
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)	SCHWALL, Ralph H.
III-4-5	Address	400 Griffin Avenue Pacifica, California 94044 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)	SLIWKOWSKI, Mark X.
III-5-5	Address	42 Oak Creek Lane San Carlos, California 94070 United States of America
III-5-6	State of nationality	US
III-5-7	State of residence	US

P2094R1

PCT REQUEST

Original (for SUBMISSION)

III-6	Applicant and/or inventor	
III-6-1	This person is	applicant and inventor
III-6-2	Applicant for	US only
III-6-4	Name (LAST, First)	SPENCER, Susan D.
III-6-5	Address	2395 Paradise Drive Tiburon, CA, California 94920 United States of America
III-6-6	State of nationality	US
III-6-7	State of residence	US
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)	ANDRUS, Alex
IV-1-2	Address	Genentech, Inc. MS 49 1 DNA Way South San Francisco, California 94080 United States of America
IV-1-3	Telephone No.	650-467-4255
IV-1-4	Facsimile No.	650-952-9881
IV-1-6	Agent's registration No.	44,509
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.	
VI-1	Priority claim of earlier national application	
VI-1-1	Filing date	01 June 2004 (01.06.2004)
VI-1-2	Number	60/576,517
VI-1-3	Country	US
VI-2	Priority claim of earlier national application	
VI-2-1	Filing date	05 October 2004 (05.10.2004)
VI-2-2	Number	60/616,098
VI-2-3	Country	US

PCT REQUEST

Original (for SUBMISSION)

VI-3	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	
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
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)	5	✓
IX-2	Description	81	-
IX-3	Claims	13	-
IX-4	Abstract	1	✓
IX-5	Drawings	15	-
IX-7	TOTAL	115	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	✓	-
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		
X-1-1	Name (LAST, First)	ANDRUS, Alex	
X-1-2	Name of signatory		
X-1-3	Capacity		

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	

ANTIBODY-DRUG CONJUGATES AND METHODS

This non-provisional application filed under 37 CFR §1.53(b), claims the benefit under 35 USC §119(e) of US Provisional Application Ser. No. 60/576, 517 filed on June 1, 2004 and US Provisional Application Ser. No. 60/616, 098 filed on October 5, 2004, each of which are incorporated by reference in their entirety.

FIELD OF THE INVENTION

The invention relates generally to compounds with anti-cancer activity and more specifically to antibodies conjugated with chemotherapeutic maytansinoid drugs or toxins. The invention also relates to methods of using antibody-drug conjugate compounds for *in vitro*, *in situ*, and *in vivo* diagnosis or treatment of mammalian cells, or associated pathological conditions.

BACKGROUND OF THE INVENTION

Antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders. The use of antibody-drug conjugates (ADC), i.e. immunoconjugates, for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells in the treatment of cancer (Payne, G. (2003) Cancer Cell 3:207-212; Trail et al (2003) Cancer Immunol. Immunother. 52:328-337; Syrigos and Epenetos (1999) Anticancer Research 19:605-614; Niculescu-Duvaz and Springer (1997) Adv. Drug Del. Rev. 26:151-172; US 4975278) theoretically 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. (eds), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Efforts to design and refine ADC have focused on the selectivity of monoclonal antibodies (mAbs) as well as drug-linking and drug-releasing properties. 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, mitomycin, neocarzinostatin (Takahashi et al (1988) Cancer 61:881-888) 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 (US 4753894; US 5629197; US 4958009; US 4956453), small molecule toxins such as geldanamycin (Mandler et al (2000) J. 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

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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.

An antibody-radioisotope conjugate has been approved, ZEVALIN® (ibritumomab tiuxetan, Biogen/Idec) composed of a murine IgG1 kappa monoclonal antibody directed against CD20 antigen and ^{111}In or ^{90}Y radioisotope bound by a thiourea linker-chelator (Wiseman et al (2000) Eur. J. 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). 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 4970198; US 5079233; US 5585089; US 5606040; US 5693762; US 5739116; US 5767285; US 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 (Xie et al (2004) J. of Pharm. and Exp. Ther. 308(3):1073-1082; Tolcher et al (2003) J. Clin. Oncology 21(2):211-222; US 5208020), underwent Phase I trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.) is an antibody-drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, 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" (also, see: US 4981979). HERCEPTIN® (trastuzumab) linked by SMCC to DM1 has been reported (WO 2005/037992).

In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides, i.e. tumor-associated antigens (TAA), has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Monoclonal antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders. An example of successful antibody therapy is HERCEPTIN® (trastuzumab), a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd = 5 nM) to the extracellular domain of the human epidermal growth factor receptor2 protein, HER2 (ErbB2) (US 5821337; US 6054297; US 6407213; US 6639055; Coussens L, et al (1985) Science 230:1132-9; Slamon DJ, et al (1989) Science 244:707-12). Trastuzumab is an IgG1 kappa antibody that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. Trastuzumab binds to the HER2 antigen and thus inhibits the growth of cancerous cells. Because trastuzumab is a humanized antibody, it minimizes any HAMA response in patients. The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary, CHO) suspension culture. The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of

185kDa, which is structurally related to the epidermal growth factor receptor. HER2 protein overexpression is observed in 25%–30% of primary breast cancers and can be determined using an immunohistochemistry based assessment of fixed tumor blocks (Press MF, *et al* (1993) Cancer Res 53:4960-70. Trastuzumab has been shown, in both *in vitro* assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2 (Hudziak RM, et al (1989) Mol Cell Biol 9:1165-72; Lewis GD, et al (1993) Cancer Immunol Immunother; 37:255-63; Baselga J, et al (1998) Cancer Res. 58:2825-2831). Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity, ADCC (Hotaling TE, et al (1996) [abstract]. Proc. Annual Meeting Am Assoc Cancer Res; 37:471; Pegram MD, et al (1997) [abstract]. Proc Am Assoc Cancer Res; 38:602; Sliwkowski et al (1999) Seminars in Oncology 26(4), Suppl 12:60-70; Yarden Y. and Sliwkowski, M. (2001) Nature Reviews: Molecular Cell Biology, Macmillan Magazines, Ltd., Vol. 2:127-137). HERCEPTIN® is clinically active in patients with ErbB2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga et al, (1996) J. Clin. Oncol. 14:737-744). Although HERCEPTIN is a breakthrough in treating patients with ErbB2-overexpressing breast cancers that have received extensive prior anti-cancer therapy, the majority of the patients in this population fail to respond or respond only poorly to HERCEPTIN treatment. Therefore, there is a significant clinical need for developing further HER2-directed cancer therapies for those patients with HER2-overexpressing tumors or other diseases associated with HER2 expression that do not respond, or respond poorly, to HERCEPTIN treatment. In addition to HER2, there is an opportunity to exploit other tumor-associated antigens with targeted therapies.

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SUMMARY

The present invention provides novel compounds with biological activity against cancer cells. The compounds may inhibit tumor growth in mammals and may be useful for treating human cancer patients.

The present invention relates to the delivery, transport, accumulation or retention of therapeutic antibody-drug conjugate (ADC) compounds inside cells. The invention is more particularly related to attaining high concentrations of active metabolite molecules in cancer cells. Intracellular targeting may be achieved by methods and compounds which allow accumulation or retention of biologically active agents inside cells. Such effective targeting may be applicable to a variety of therapeutic formulations and procedures.

The surprising discovery has been made that antibody-drug conjugates with stable, non-disulfide linkers groups that attach a maytansinoid drug moiety to an antibody result in increased *in vitro* potency and *in vivo* efficacy. In addition, the antibody-drug conjugates show the unexpected result of better safety *in vivo* relative to certain disulfide linker conjugates.

Antibody-drug conjugate (ADC) compounds comprise an antibody covalently attached by a linker to one or more maytansinoid drug moieties. ADC may be represented by Formula I:

$$Ab–(L–D)_p \qquad \text{I}$$

where one or more maytansinoid drug moieties (D) are covalently linked by L to an antibody (Ab). Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors. The linker L may be stable outside a cell, i.e. extracellular. The linker L, the maytansinoid drug moiety D, or the linker and the maytansinoid drug moiety taken together (L-D), do not comprise a disulfide group.

In one embodiment, a substantial amount of the drug moiety is not cleaved from the antibody until the antibody-drug conjugate enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate, and the drug moiety is cleaved from the antibody when the antibody-drug conjugate does enter the cell.

In another embodiment, the ADC specifically binds to a receptor encoded by an ErbB gene, such as EGFR, HER2, HER3 and HER4. The ADC may specifically bind to the extracellular domain of the HER2 receptor. The ADC may inhibit growth of tumor cells which overexpress HER2 receptor.

In another embodiment, the antibody (Ab) of Formula I is a humanized antibody such as huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 or huMAb4D5-8 (trastuzumab).

Another aspect of the invention is a pharmaceutical composition including a Formula I compound, or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable diluent, carrier, or excipient.

Another aspect provides a pharmaceutical combination comprising a Formula I compound and a second compound having anti-cancer properties or other therapeutic effects..

Another aspect includes diagnostic and therapeutic uses for the compounds and compositions disclosed herein.

Another aspect is a method for killing or inhibiting the proliferation of tumor cells or cancer cells comprising treating the cells with an amount of an antibody-drug conjugate, or a pharmaceutically acceptable salt or solvate thereof, being effective to kill or inhibit the proliferation of the tumor cells or cancer cells.

Another aspect are methods of treating cancer comprising administering to a patient a formulation of a Formula I compound. One method is for the treatment of cancer in a mammal, wherein the cancer is characterized by the overexpression of an ErbB receptor. The mammal optionally does not respond, or responds poorly, to treatment with an unconjugated anti-ErbB antibody. The method comprises administering to the mammal a therapeutically effective amount of an antibody-drug conjugate compound.

Another aspect is a method of inhibiting the growth of tumor cells that overexpress a growth factor receptor selected from the group consisting of HER2 receptor and EGF receptor comprising administering to a patient an antibody-drug conjugate compound which binds specifically to said growth factor receptor and a chemotherapeutic agent wherein said antibody-drug conjugate and said chemotherapeutic agent are each administered in amounts effective to inhibit growth of tumor cells in the patient.

Another aspect is a method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor, comprising administering a combination of an antibody-drug conjugate compound of Formula I and a chemotherapeutic agent.

Another aspect is an assay method for detecting cancer cells comprising: exposing cells to an antibody-drug conjugate compound, and determining the extent of binding of the antibody-drug conjugate compound to the cells.

Another aspect concerns methods of screening ADC drug candidates for the treatment of a disease or disorder where the disease or disorder is characterized by the overexpression of HER2.

Another aspect includes articles of manufacture, i.e. kits, comprising an antibody-drug conjugate, a container, and a package insert or label indicating a treatment.

Another aspect includes methods of treating a disease or disorder characterized by the overexpression of HER2 in a patient with the antibody-drug conjugate compounds.

Another aspect includes methods of making, methods of preparing, methods of synthesis, methods of conjugation, and methods of purification of the antibody-drug conjugate compounds, and the intermediates for the preparation, synthesis, and conjugation of the antibody-drug conjugate compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an *in vitro*, cell proliferation assay with SK-BR-3 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 2 shows an *in vitro*, cell proliferation assay with BT-474 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 3 shows an *in vitro*, cell proliferation assay with MCF7 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 4 shows an *in vitro*, cell proliferation assay with MDA-MB-468 cells treated with antibody-drug conjugates: –□– trastuzumab-SPP-DM1, –Δ– trastuzumab-SPDP-DM1, and –o– trastuzumab-SMCC-DM1.

Figure 5 shows the serum clearance in beige nude mice without tumors of trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, measuring conjugate and total antibody serum concentration at six time points (5 minutes, 1 hour, 6 hours, 24 hours, 72, 168 hours post-dose) over 7 days.

Figure 6 shows the stability over time in nude mice without tumors of the conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, and trastuzumab-SMCC-DM1, measuring serum concentration at six time points (5 minutes, 1 hour, 6 hours, 24 hours, 72, 168 hours post-dose) over 7 days.

Figure 7 shows the measurement of serum concentrations of total trastuzumab / trastuzumab-SMCC-DM1, and total trastuzumab / trastuzumab-SPP-DM1 in mice, 7 days after treatment, with and without tumor.

Figure 8 shows a plasma concentration clearance study after administration of 10 mg/kg of trastuzumab-SPP-DM1 to 4 subject rats. Concentrations of total antibody and trastuzumab-SPP-DM1 were measured. (tr = trastuzumab)

Figure 9 shows a plasma concentration clearance study after administration of 10 mg/kg of trastuzumab-SMCC-DM1 to 4 subject rats. Concentrations of total antibody and trastuzumab-SMCC-DM1 were measured.

Figure 10 shows the mean tumor volume change over time in mice dosed with: Vehicle (PBS pH 6.5), trastuzumab-SPP-DM1 (370 μg DM1/m^2), and trastuzumab-SMCC-DM1 (330 μg DM1/m^2) where dose refers to the dose of DM1 administered.

Figure 11 shows the mean tumor volume change over time in athymic nude mice with Fo5 tumor allografts dosed on Day 0 with: Vehicle (PBS pH 6.5), 10 mg/kg trastuzumab-SIAB-DM1 (3.4 DM1/Ab; 168 μg DM1/kg), and 10 mg/kg trastuzumab-SMCC-DM1 (3.2 DM1/Ab; 158 μg DM1/kg) where dose refers to the dose amount of the antibody-drug conjugate administered.

Figure 12 shows the mean tumor volume change over time in MMTV-Her2 Fo 5 beige nude mice (seven each group, all with tumors, Ti = 7) by single injection with Vehicle (PBS pH 6.5), 10 mg/kg trastuzumab-

SPP-DM1, 10 mg/kg trastuzumab-SPP-DM4, 10 mg/kg trastuzumab-SPP-DM3, and 10 mg/kg trastuzumab-SMCC-DM1.

Figure 13 shows the time to double tumor volume and log cell kill analysis for Vehicle (PBS pH 6.5), trastuzumab-SPP-DM1, trastuzumab-SPP-DM4, trastuzumab-SPP-DM3, and trastuzumab-SMCC-DM1 in HER2-Fo5 tumors.

Figure 14 shows the change in body weight over time of rats dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (1860 μg DM1/m^2), trastuzumab-SMCC-DM1 (3260 μg DM1/m^2), and free DM1 (650 μg/m^2).

Figure 15 shows a liver function test measured in AST units per liter over time in the rat model dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), and free DM1.

Figure 16 shows a safety profile measured in PLT units in cells per liter over time in the rat model dosed with: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0), trastuzumab-SPP-DM1 (22.3 mg/kg), trastuzumab-SMCC-DM1 (10 mg/kg), trastuzumab-SMCC-DM1 (25 mg/kg), trastuzumab-SMCC-DM1 (50 mg/kg), and free DM1.

Figure 17 shows an in vitro, cell proliferation assay with HT1080EphB2 (C8) cells treated with antibody-drug conjugates: –▲– antiEphB2R 2H9-SPP-DM1, and –▼– antiEphB2R 2H9-SMCC-DM1.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs, and are consistent with: Singleton *et al.*, (1994) *Dictionary of Microbiology and Molecular Biology, 2nd Ed.*, J. Wiley & Sons, New York, NY; and Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity (Miller et al (2003) Jour. of Immunology 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology, 5th Ed.*, Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, *i.e.*, a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (*e.g.*, IgG, IgE, IgM, IgD, and IgA), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, US 4816567). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597; for example.

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 (US 4816567; and Morrison et al (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey or Ape) and human constant region sequences.

An "intact antibody" herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more "effector functions" which 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. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

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

An "ErbB receptor" is a receptor protein tyrosine kinase which belongs to the ErbB receptor family which are important mediators of cell growth, differentiation and survival. The ErbB receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or $p185^{neu}$), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). A panel of anti-ErbB2 antibodies has been characterized using the human breast tumor cell line SKBR3 (Hudziak et al (1989) Mol. Cell. Biol. 9(3):1165-1172. Maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α (US 5677171). The anti-ErbB2 antibodies discussed in Hudziak et al are further characterized in Fendly et al (1990) Cancer Research 50:1550-1558; Kotts et al. (1990) In Vitro 26(3):59A; Sarup et al. (1991) Growth Regulation 1:72-82; Shepard et al. J. (1991) Clin. Immunol. 11(3):117-127; Kumar et al. (1991) Mol. Cell. Biol. 11(2):979-986; Lewis et al. (1993) Cancer Immunol. Immunother. 37:255-263; Pietras et al. (1994) Oncogene 9:1829-1838; Vitetta et al. (1994) Cancer Research 54:5301-5309; Sliwkowski et al. (1994) J. Biol. Chem. 269(20):14661-14665; Scott et al. (1991) J. Biol. Chem. 266:14300-5; D'souza et al. Proc. Natl. Acad. Sci. (1994) 91:7202-7206; Lewis et al. (1996) Cancer Research 56:1457-1465; and Schaefer et al. (1997) Oncogene 15:1385-1394.

Other anti-ErbB2 antibodies with various properties have been described in Franklin et al (2004) Cancer Cell 5:317-328; Tagliabue et al (1991) Int. J. Cancer 47:933-937; McKenzie et al (1989) Oncogene 4:543-548; Maier et al (1991) Cancer Res. 51:5361-5369; Bacus et al (1990) Molecular Carcinogenesis 3:350-362; Stancovski et al (1991) PNAS (USA) 88:8691-8695; Bacus et al (1992) Cancer Research 52:2580-2589; Xu et al (1993) Int. J. Cancer 53:401-408; WO94/00136; Kasprzyk et al (1992) Cancer Research 52:2771-2776;

Hancock et al (1991) Cancer Res. 51:4575-4580; Shawver et al (1994) Cancer Res. 54:1367-1373; Arteaga et al (1994) Cancer Res. 54:3758-3765; Harwerth et al (1992) J. Biol. Chem. 267:15160-15167; US 5783186; and Klapper et al (1997) Oncogene 14:2099-2109.

Sequence identity screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (US 5,183,884; US 5,480,968; Kraus et al (1989) PNAS (USA) 86:9193-9197) and ErbB4 (EP 599274; Plowman et al (1993) Proc. Natl. Acad. Sci. USA, 90:1746-1750; and Plowman et al (1993) Nature 366:473-475). Both of these receptors display increased expression on at least some breast cancer cell lines.

The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a "native sequence" ErbB receptor or an "amino acid sequence variant" thereof. The ErbB receptor may be native sequence human ErbB receptor. Accordingly, a "member of the ErbB receptor family" is EGFR (ErbB1), ErbB2, ErbB3, ErbB4 or any other ErbB receptor currently known or to be identified in the future.

The terms "ErbB1", "epidermal growth factor receptor", "EGFR" and "HER1" are used interchangeably herein and refer to EGFR as disclosed, for example, in Carpenter et al (1987) Ann. Rev. Biochem. 56:881-914, including naturally occurring mutant forms thereof (e.g., a deletion mutant EGFR as in Humphrey et al., (1990) PNAS (USA), 87:4207-4211). The term erbB1 refers to the gene encoding the EGFR protein product. Antibodies against HER1 are described, for example, in Murthy et al (1987) Arch. Biochem. Biophys., 252:549-560 and in WO 95/25167.

The term "ERRP", "EGF-Receptor Related Protein", "EGFR Related Protein" and "epidermal growth factor receptor related protein" are used interchangeably herein and refer to ERRP as disclosed, for example in US 6399743 and US 2003/0096373.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al (1985) PNAS (USA), 82:6497-6501 and Yamamoto et al (1986) Nature, 319:230-234 (Genbank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat p185neu.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in US 5183884; US 5480968; Kraus et al (1989) PNAS (USA) 86:9193-9197. Antibodies against ErbB3 are known in the art (US 5183884; US 5480968; WO 97/35885).

The terms "ErbB4" and "HER4" herein refer to the receptor polypeptide as disclosed, for example, in EP Pat Appln No 599,274; Plowman et al., Proc. Natl. Acad. Sci. USA, 90:1746-1750 (1993); and Plowman et al., Nature, 366:473-475 (1993), including isoforms thereof, e.g., as disclosed in WO 99/19488. Antibodies against HER4 are described, for example, in WO 02/18444.

Antibodies to ErbB receptors are available commercially from a number of sources, including, for example, Santa Cruz Biotechnology, Inc., California, USA.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand may be a native sequence human ErbB ligand such as epidermal growth factor (EGF) (Savage et al (1972) J. Biol. Chem., 247:7612-7621); transforming growth factor alpha (TGF-α) (Marquardt et al (1984) Science 223:1079-1082); amphiregulin also known as schwanoma or keratinocyte autocrine growth factor (Shoyab et al (1989) Science 243:1074-1076; Kimura et al (1990) Nature 348:257-260; and Cook et al (1991)

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Mol. Cell. Biol., 11:2547-2557); betacellulin (Shing et al (1993) Science 259:1604-1607; and Sasada et al (1993) Biochem. Biophys. Res. Commun. 190:1173); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama et al (1991) Science 251:936-939); epiregulin (Toyoda et al (1995) J. Biol. Chem. 270:7495-7500; and Komurasaki et al (1997) Oncogene 15:2841-2848); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway et al., Nature, 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang et al (1997) Proc. Natl. Acad. Sci., 94:9562-9567); neuregulin-4 (NRG-4) (Harari et al (1999) Oncogene, 18:2681-89) or cripto (CR-1) (Kannan et al (1997) J. Biol. Chem., 272(6):3330-3335). ErbB ligands which bind EGFR include EGF, TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins. The ErbB ligand may also be a synthetic ErbB ligand. The synthetic ligand may be specific for a particular ErbB receptor, or may recognize particular ErbB receptor complexes. An example of a synthetic ligand is the synthetic heregulin/EGF chimera biregulin (see, for example, Jones et al (1999) FEBS Letters, 447:227-231, which is incorporated by reference).

"Heregulin" (HRG) refers to a polypeptide encoded by the heregulin gene product as disclosed in US 5641869 or Marchionni et al (1993) Nature 362:312-318. Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes et al (1992) Science 256:1205-1210; and US 5641869); neu differentiation factor (NDF) (Peles et al (1992) Cell 69: 205-216); acetylcholine receptor-inducing activity (ARIA) (Falls et al (1993) Cell 72:801-815); glial growth factors (GGFs) (Marchionni et al (1993) Nature, 362:312-318); sensory and motor neuron derived factor (SMDF) (Ho et al (1995) J. Biol. Chem. 270:14523-14532); γ-heregulin (Schaefer et al (1997) Oncogene, 15:1385-1394). The term includes biologically active fragments and/or amino acid sequence variants of a native sequence HRG polypeptide, such as an EGF-like domain fragment thereof (*e.g.*, HRGβ1177-244).

"ErbB hetero-oligomer" is a noncovalently associated oligomer comprising at least two different ErbB receptors. An "ErbB dimer" is a noncovalently associated oligomer that comprises two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand. ErbB oligomers, such as ErbB dimers, can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al (1994) J. Biol. Chem., 269(20):14661-14665, for example. Examples of such ErbB hetero-oligomers include EGFR-ErbB2 (also referred to as HER1/HER2), ErbB2-ErbB3 (HER2/HER3) and ErbB3-ErbB4 (HER3/HER4) complexes. Moreover, the ErbB hetero-oligomer may comprise two or more ErbB2 receptors combined with a different ErbB receptor, such as ErbB3, ErbB4 or EGFR (ErbB1). Other proteins, such as a cytokine receptor subunit (*e.g.*, gp130) may be included in the hetero-oligomer.

By "ligand activation of an ErbB receptor" is meant signal transduction (e.g., that caused by an intracellular kinase domain of an ErbB receptor phosphorylating tyrosine residues in the ErbB receptor or a substrate polypeptide) mediated by ErbB ligand binding to a ErbB hetero-oligomer comprising the ErbB receptor of interest. Generally, this will involve binding of an ErbB ligand to an ErbB hetero-oligomer which activates a kinase domain of one or more of the ErbB receptors in the hetero-oligomer and thereby results in phosphorylation of tyrosine residues in one or more of the ErbB receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s). ErbB receptor activation can be quantified using various tyrosine phosphorylation assays.

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A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (e.g. ErbB receptor or ErbB ligand) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants will possess at least about 70% sequence identity with at least one receptor binding domain of a native ErbB ligand or with at least one ligand binding domain of a native ErbB receptor, or at least about 80%, or at least about 90% homologous with such receptor or ligand binding domains. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

"Sequence identity" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2," authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, DC 20559, on December 10, 1991.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, (1991) Annu. Rev. Immunol, 9:457-92. To assess ADCC activity of a molecule of interest, an in vitro ADCC assay may be performed (US 5500362l; US 5821337). 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 (1998) PNAS (USA), 95:652-656.

"Maytansinoid drug moiety" means the substructure of an antibody-drug conjugate that has the structure of a maytansine compound. Maytansine was first isolated from the east African shrub *Maytenus serrata* (US 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (US 4151042). Synthetic maytansinol and maytansinol analogues have been reported. See US Patent Nos. 4137230; 4248870; 4256746; 4260608; 4265814; 4294757; 4307016; 4308268; 4308269; 4309428; 4313946; 4315929; 4317821; 4322348; 4331598; 4361650; 4364866; 4424219; 4450254; 4362663; and 4371533, and Kawai et al (1984) Chem. Pharm. Bull. 3441-3451), each of which are expressly incorporated by reference.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody, such as a native sequence human FcR. FcR may bind an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these 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

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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 review M. in 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 (1994) Immunomethods, 4:25-34; and de Haas et al (1995) J. Lab. Clin. Med., 126:330-41. Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al (1976) J. Immunol. 117:587, and Kim et al (1994) J. Immunol., 24:249).

"Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (*e.g.*, an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al (1996) J. Immunol. Methods, 202:163, may be performed.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

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

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al *supra*) and/or those residues from a "hypervariable loop" (*e.g.*, residues 26-32 (L1), 50-52

(L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Leak (1987) J. Mol. Biol., 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

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

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies 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.

"Single-chain Fv" or "scFv" mean single chain variable region antibody fragments which comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. The Fv polypeptide may further comprise a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding (Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). Anti-ErbB2 antibody scFv fragments are described in WO 93/16185; US 5571894; US 5587458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (VH) connected to a variable light 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 are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448.

"Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues.

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Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al (1986) Nature, 321:522-525; Riechmann et al (1988) Nature 332:323-329; and Presta, (1992) Curr. Op. Struct. Biol., 2:593-596.

Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®, trastuzumab) as described in Table 3 of US 5821337 expressly incorporated herein by reference; humanized 520C9 (WO 93/21319) and humanized 2C4 antibodies.

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 diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. The antibody may be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, or more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup protein sequencer, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using 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 antibody "which binds" an antigen of interest, *e.g.*, ErbB2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. Where the antibody is one which binds ErbB2, it will usually preferentially bind ErbB2 as opposed to other ErbB receptors, and may be one which does not significantly cross-react with other proteins such as EGFR, ErbB3 or ErbB4. In such embodiments, the extent of binding of the antibody to these non-ErbB2 proteins (*e.g.*, cell surface binding to endogenous receptor) will be less than 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). Sometimes, the anti-ErbB2 antibody will not significantly cross-react with the rat neu protein, *e.g.*, as described in Schecter et al (1984) Nature 312:513, and Drebin et al (1984) Nature, 312:545-548.

An antibody which "blocks" ligand activation of an ErbB receptor reduces or prevents such activation, wherein the antibody is able to block ligand activation of the ErbB receptor substantially more effectively than monoclonal antibody 4D5, *e.g.*, about as effectively as monoclonal antibodies 7F3 or 2C4 or Fab fragments thereof. For example, the antibody that blocks ligand activation of an ErbB receptor may be one which is about 50-100% more effective than 4D5 at blocking formation of an ErbB hetero-oligomer. Blocking of ligand activation of an ErbB receptor can occur by any means, *e.g.*, by interfering with: ligand binding to an ErbB receptor, ErbB complex formation, tyrosine kinase activity of an ErbB receptor in an ErbB complex and/or phosphorylation of tyrosine kinase residue(s) in or by an ErbB receptor.

An antibody having a "biological characteristic" of a designated antibody, such as the monoclonal antibody designated 2C4 (Omnitarg, Genentech, Inc.), is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen (*e.g.*, ErbB2). For example, an antibody with a biological characteristic of 2C4 may block HRG activation of an ErbB hetero-oligomer comprising ErbB2 and ErbB3, ErbB1 or ErbB4; block EGF, TGF-α, HB-EGF, epiregulin and/or amphiregulin activation of an ErbB receptor comprising EGFR and ErbB2; block EGF, TGF-α and/or HRG mediated activation of MAPK; and/or bind the same epitope in the extracellular domain of ErbB2 as that bound by 2C4 (*e.g.*, which blocks binding of monoclonal antibody 2C4 to ErbB2).

Unless indicated otherwise, the expression "monoclonal antibody 2C4" refers to an antibody that has antigen binding residues of, or derived from, the murine 2C4 antibody of the Examples below. For example, the monoclonal antibody 2C4 may be murine monoclonal antibody 2C4 or a variant thereof, such as humanized antibody 2C4, possessing antigen binding amino acid residues of murine monoclonal antibody 2C4 (WO 01/00245). Unless indicated otherwise, the expression "rhuMAb 2C4" when used herein refers to an antibody comprising the variable light (VL) and variable heavy (VH) sequences of SEQ ID Nos. 3 and 4, respectively, fused to human light and heavy IgG1 (non-A allotype) constant region sequences optionally expressed by a Chinese Hamster Ovary (CHO) cell (WO 01/00245).

Unless indicated otherwise, the term "monoclonal antibody 4D5" refers to an antibody that has antigen binding residues of, or derived from, the murine 4D5 antibody (ATCC CRL 10463). For example, the monoclonal antibody 4D5 may be murine monoclonal antibody 4D5 or a variant thereof, such as a humanized 4D5, possessing antigen binding residues of murine monoclonal antibody 4D5. Exemplary humanized 4D5 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as in US 5821337.

A "growth inhibitory agent" refers to a compound or composition which inhibits growth of a cell, e.g. an ErbB expressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of ErbB expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest (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). Examples of "growth inhibitory" antibodies are those which bind to ErbB2 and inhibit the growth of cancer cells overexpressing ErbB2. Growth inhibitory anti-ErbB2 antibodies may inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, or greater than 50% (e.g., from about 50% to about 100%) at an antibody concentration of about 0.5 to 30 μg/ml, where the growth inhibition is determined six days after exposure of the SK-BR-3 cells to the antibody (US 5677171).

An antibody which "induces cell death" is one which causes a viable cell to become nonviable. The cell is generally one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. The cell may be a cancer cell, *e.g.*, a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death *in vitro* may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using

heat inactivated serum (*i.e.*, in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al (1995) Cytotechnology, 17:1-11) or 7AAD can be assessed relative to untreated cells. Cell death-inducing antibodies are those which induce PI uptake in the PI uptake assay in BT474 cells (see below).

An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses the ErbB2 receptor, including a tumor cell, *e.g.*, a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell.

The terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "disorder" is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemia and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders. An exemplary disorder to be treated in accordance with the present invention is a solid, malignant tumor

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may: (i) reduce the number of cancer cells; (ii) reduce the tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; and/or (vi) relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. In animal models, efficacy may be assessed by physical measurements of the tumor during the course following administration of the ADC, and by determining partial and complete remission of tumor. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

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

An "ErbB-expressing cancer" is one comprising cells which have ErbB protein present at their cell surface. An "ErbB2-expressing cancer" is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer.

A cancer which "overexpresses" a receptor, e.g. an ErbB receptor, is one which has significantly higher levels of the receptor, such as ErbB2, at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB protein present on the surface of a cell (*e.g.*, via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB-encoding nucleic acid in the cell, *e.g.*, via fluorescent in situ hybridization (FISH; see WO 98/45479), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Overexpression of the ErbB ligand, may be determined diagnostically by evaluating levels of the ligand (or nucleic acid encoding it) in the patient, *e.g.*, in a tumor biopsy or by various diagnostic assays such as the IHC, FISH, southern blotting, PCR or in vivo assays described above. One may also study ErbB receptor overexpression by measuring shed antigen (*e.g.*, ErbB extracellular domain) in a biological fluid such as serum (see, *e.g.*, US 4933294; WO 91/05264; US 5401638; and Sias et al (1990) J. Immunol. Methods 132: 73-80). Aside from the above assays, various other in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.*, a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.*, by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The tumors overexpressing HER2 are rated by immunohistochemical scores corresponding to the number of copies of HER2 molecules expressed per cell, and can be determined biochemically: 0 = 0-10,000 copies/cell, 1+ = at least about 200,000 copies/cell, 2+ = at least about 500,000 copies/cell, 3+ = about 1-2 x 10^6 copies/cell. Overexpression of HER2 at the 3+ level, which leads to ligand-independent activation of the tyrosine kinase (Hudziak et al., (1987) Proc. Natl. Acad. Sci. USA 84:7159-7163), occurs in approximately 30% of breast cancers, and in these patients, relapse-free survival and overall survival are diminished (Slamon et al

(1989) Science 244:707-712; Slamon et al (1987) Science, 235:177-182). Conversely, a cancer which is "not characterized by overexpression of the ErbB2 receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB2 receptor compared to a noncancerous cell of the same tissue type. The murine monoclonal anti-HER2 antibody inhibits the growth of breast cancer cell lines that overexpress HER2 at the 2+ and 3+ (1-2 x 10^6 HER2 receptors per cell) level, but has no activity on cells that express lower levels of HER2 (Lewis et al (1993) Cancer Immunol. Immunother. 37:255-263). Based on this observation, antibody 4D5 was humanized (huMAb4D5-8, rhuMAb HER2, US 5821337; Carter et al (1992) Proc. Natl. Acad. Sci. USA 89: 4285-4289) and tested in breast cancer patients whose tumors overexpress HER2 but who had progressed after conventional chemotherapy (Cobleigh et al (1999) J. Clin. Oncol. 17: 2639-2648). Most patient tumors in this trial expressed HER2 at the 3+ level, though a fraction was 2+.

A "hormone independent" cancer is one in which proliferation thereof is not dependent on the presence of a hormone which binds to a receptor expressed by cells in the cancer. Such cancers do not undergo clinical regression upon administration of pharmacological or surgical strategies that reduce the hormone concentration in or near the tumor. Examples of hormone independent cancers include androgen independent prostate cancer, estrogen independent breast cancer, endometrial cancer and ovarian cancer. Such cancers may begin as hormone dependent tumors and progress from a hormone-sensitive stage to a hormone-refractory tumor following anti-hormonal therapy.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.*, ^{211}At, ^{131}I, ^{125}I, ^{90}Y, ^{186}Re, ^{188}Re, ^{153}Sm, ^{212}Bi, ^{32}P, ^{60}C, and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogs and derivatives thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Erlotinib (TARCEVA®, Genentech/OSI Pharm.), Bortezomib (VELCADE®, Millenium Pharm.), Fulvestrant (FASLODEX®, Astrazeneca), Sutent (SU11248, Pfizer), Letrozole (FEMARA®, Novartis), Imatinib mesylate (GLEEVEC®, Novartis), PTK787/ZK 222584 (Novartis), Oxaliplatin (Eloxatin®, Sanofi), 5-FU (5-fluorouracil), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (GSK572016, GlaxoSmithKline), Lonafarnib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs.), and Gefitinib (IRESSA®, Astrazeneca), AG1478, AG1571 (SU 5271; Sugen), 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 trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); 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; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g.,

calicheamicin, especially calicheamicin gamma1I and calicheamicin omega1I (Angew Chem Intl. Ed. Engl. (1994) 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, 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, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; 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; podophyllinic acid; 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; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; 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® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition of "chemotherapeutic agent" are: (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON· toremifene; (ii) 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; (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) aromatase inhibitors; (v) protein kinase inhibitors; (vi) lipid kinase

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inhibitors; (vii) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (viii) ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME® ribozyme) and a HER2 expression inhibitor; (ix) vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; (x) anti-angiogenic agents such as bevacizumab (AVASTIN®, Genentech); and (xi) pharmaceutically acceptable salts, acids or derivatives of any of the above.

Protein kinase inhibitors include tyrosine kinase inhibitors which inhibit to some extent tyrosine kinase activity of a tyrosine kinase such as an ErbB receptor. Examples of tyrosine kinase inhibitors include EGFR-targeted drugs such as: (i) antibodies which bind to EGFR, including MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US 4943533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBITUX®, Imclone) and reshaped human 225 (H225) (WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (US 5212290); humanized and chimeric antibodies that bind EGFR (US 5891996); and human antibodies that bind EGFR, such as ABX-EGF (WO 98/50433); (ii) anti-EGFR antibody conjugated with a cytotoxic agent (EP 659439A2); and small molecules that bind to EGFR including ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen), quinazolines such as PD 153035,4-(3-chloroanilino) quinazoline, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706, and pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines, curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide), tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (*e.g.*, those that bind to ErbB-encoding nucleic acid); quinoxalines (US 5804396); tryphostins (US 5804396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-ErbB inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (Gleevac; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxanib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in: US 5804396; WO 99/09016 (American Cyanamid); WO 98/43960 (American Cyanamid); WO 97/38983 (Warner Lambert); WO 99/06378 (Warner Lambert); WO 99/06396 (Warner Lambert); WO 96/30347 (Pfizer, Inc); WO 96/33978 (Zeneca); WO 96/3397 (Zeneca); and WO 96/33980 (Zeneca).

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. An exemplary anti-angiogenic agent is an antibody that binds to Vascular Endothelial Growth Factor (VEGF) such as bevacizumab (AVASTIN®, Genentech).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor;

prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

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

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-ErbB2 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

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

A "cardioprotectant" is a compound or composition which prevents or reduces myocardial dysfunction (*i.e.*, cardiomyopathy and/or congestive heart failure) associated with administration of a drug, such as an anthracycline antibiotic and/or an anti-ErbB2 antibody, to a patient. The cardioprotectant may, for example, block or reduce a free-radical-mediated cardiotoxic effect and/or prevent or reduce oxidative-stress injury. Examples of cardioprotectants encompassed by the present definition include the iron-chelating agent dexrazoxane (ICRF-187) (Seifert et al., *The Annals of Pharmacotherapy*, 28:1063-1072 (1994)); a lipid-lowering agent and/or anti-oxidant such as probucol (Singal et al., *J. Mol. Cell Cardiol.*, 27:1055-1063 (1995)); amifostine (aminothiol 2-[(3-aminopropyl)amino]ethanethiol-dihydrogen phosphate ester, also called WR-2721, and the dephosphorylated cellular uptake form thereof called WR-1065) and S-3-(3-methylaminopropylamino)propylphosphorothioic acid (WR-151327), see Green et al., (1994) Cancer Research,

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54:738-741; digoxin (Bristow, M.R. ed. (1980) *Drug-Induced Heart Disease*. New York: Elsevier 191-215); beta-blockers such as metoprolol (Hjalmarson et al (1994) Drugs 47:Suppl 4:31-9; and Shaddy et al (1995) Am. Heart J., 129:197-9); vitamin E; ascorbic acid (vitamin C); free radical scavengers such as oleanolic acid, ursolic acid and N-acetylcysteine (NAC); spin trapping compounds such as alpha-phenyl-tert-butyl nitrone (PBN); (Paracchini et al (1993) Anticancer Res., 13:1607-1612); selenoorganic compounds such as P251 (Elbesen); and the like.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

"Alkyl" is C_1-C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples of alkyl radicals include C_1-C_8 hydrocarbon moieties such as, but not limited to: methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$.

"Alkenyl" is C_2-C_{18} 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 of alkenyl radicals include C_2-C_8 hydrocarbon moieties such as, but not limited to: ethylene or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$), cyclopentenyl ($-C_5H_7$), and 5-hexenyl ($-CH_2\ CH_2CH_2CH_2CH=CH_2$)

"Alkynyl" is C_2-C_{18} 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 of alkynyl radicals include C_2-C_8 hydrocarbon moieties such as, but not limited to: acetylenic ($-C\equiv CH$) and propargyl ($-CH_2C\equiv CH$),

"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 C_1-C_8 hydrocarbon moieties such

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as, 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.

"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 C_2-C_8 hydrocarbon moieties such as, 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 C_2-C_8 hydrocarbon moieties such as, but are not limited to: acetylene (-C≡C-), propargyl (-CH_2C≡C-), and 4-pentynyl (-$CH_2CH_2CH_2$C≡C-).

"Aryl", alone or in combination, means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Aryl radical may contain one, two or three rings wherein such rings may be attached together in a pendent manner, e.g. biphenyl, or may be fused, e.g. napthalene or anthracene. Some aryl groups are represented in the exemplary structures as "Ar". Typical aryl groups include C_6-C_{12} hydrocarbon moieties such as, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"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.

Alkyl, alkylene, aryl, arylalkyl, and heteroarylalkyl groups may be substituted where one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O^-, -OR, -SR, -S^-, -NR_2, -NR_3, =NR, -CX_3, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO_2, =N_2, -N_3, -NC(=O)R, -C(=O)R, -C(=O)NR_2, -SO_3^-, -SO_3H, -S(=O)$_2$R, -OS(=O)$_2$OR, -S(=O)$_2$NR, -S(=O)R, -OP(=O)(OR)$_2$, -P(=O)(OR)$_2$, -PO^-_3, -PO_3H_2, -C(=O)R, -C(=O)X, -C(=S)R, -CO_2R, -CO_2^-, -C(=S)OR, -C(=O)SR, -C(=S)SR, -C(=O)NR_2, -C(=S)NR_2, -C(=NR)NR_2, where each X is independently a halogen: F, Cl,

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Br, or I; and each R is independently H, C_1–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", also known as heterocycle or heterocyclyl, refers to a ring system radical in which one or more ring atoms is a heteroatom, e.g. nitrogen, oxygen, and sulfur. The heteroaryl radical comprises 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. A heteroaryl 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. Heteroaryl compounds 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.

"Linker" 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. Linkers include a divalent radical such as an alkylene, 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.

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"

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and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

The phrase "pharmaceutically acceptable salt," as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an ADC. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

"Pharmaceutically acceptable solvate" refers to an association of one or more solvent molecules and an ADC. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

ANTIBODY-DRUG CONJUGATES

The compounds of the invention include those with utility for anticancer activity. In particular, the compounds include an antibody conjugated, i.e. covalently attached by a linker, to a drug moiety where the drug when not conjugated to an antibody has a cytotoxic or cytostatic effect. The biological activity of the drug moiety is thus modulated by conjugation to an antibody. The antibody-drug conjugates (ADC) of the invention may selectively deliver an effective dose of a cytotoxic agent to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose may be achieved.

In one embodiment, the bioavailability of the ADC, or an intracellular metabolite of the ADC, is improved in a mammal when compared to the corresponding maytansinoid compound alone. Also, the bioavailability of the ADC, or an intracellular metabolite of the ADC is improved in a mammal when compared to the corresponding antibody alone (antibody of the ADC, without the drug moiety or linker).

In one embodiment, the maytansinoid drug moiety of the ADC is not cleaved from the antibody until the antibody-drug conjugate binds to a cell-surface receptor or enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate. The drug moiety may be cleaved from the antibody after the antibody-drug conjugate enters the cell. The maytansinoid drug moiety may be intracellularly cleaved in a mammal from the antibody of the compound, or an intracellular metabolite of the compound, by enzymatic action, hydrolysis, oxidation, or other mechanism. For example, and in no way meant to limit the invention to a particular mechanism of action, the sulfur atom of the maytansinoid drug moiety of the ADC may be oxidized to a sulfone or sulfoxide group. Protons on carbons bound to the sulfone and sulfoxide may be removed under general or enzymatic catalysis inside the cell and result in a beta-elimination fragmentation that cleaves and separates the drug moiety from the antibody of the ADC. Alternatively, other electron withdrawing groups such as amides in the linker, antibody or drug moiety may effect similar fragmentation/cleavage mechanisms inside a cell.

Antibody-drug conjugates (ADC) may be represented by Formula I:

358 360

$$Ab–(L–D)_p \quad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

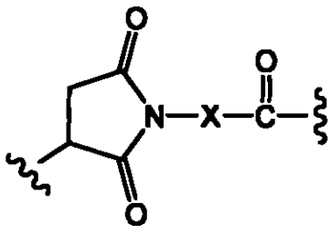
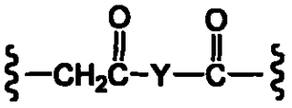
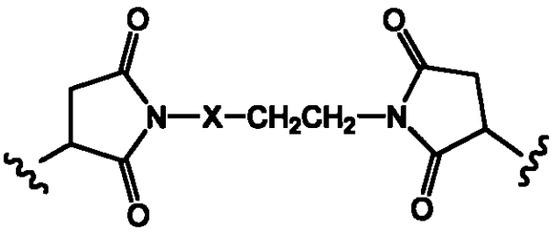
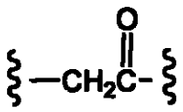
(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

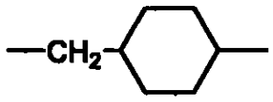
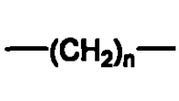
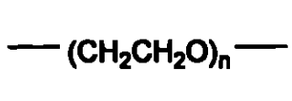
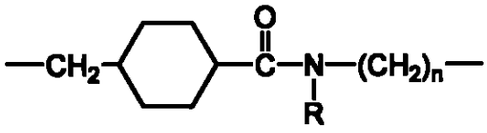
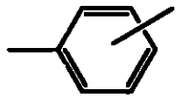
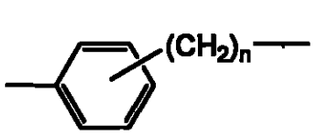
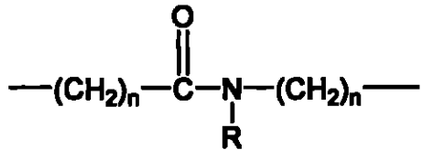
provided that the antibody is not TA.1.

L is a non-disulfide linker. L includes but is not limited to the structures:

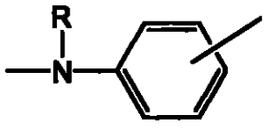
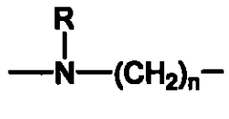





where the wavy lines indicate the covalent attachments to Ab and D;

X is:

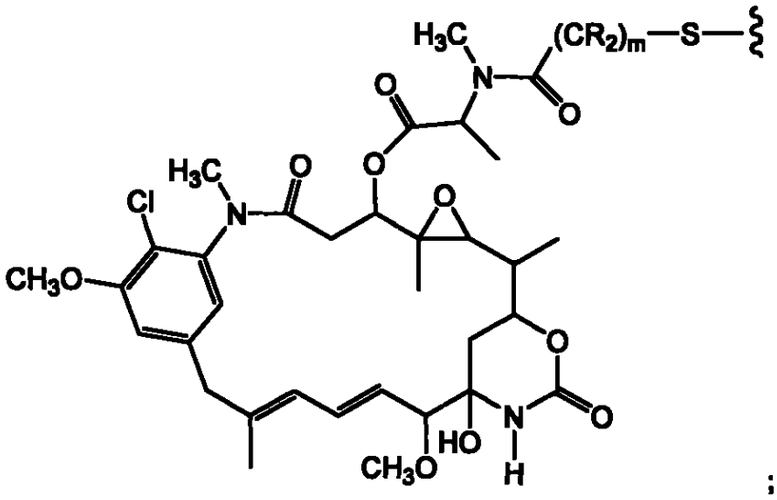








Y is:

R is independently H or C_1–C_6 alkyl; and n is 1 to 12;

D is a maytansinoid drug moiety. Maytansinoids include, but are not limited to the structure:



where the wavy line indicates the covalent attachment to L;

R is independently H or C_1–C_6 alkyl; and

m is 1, 2, or 3.

The drug to antibody ratio or drug loading is represented by p for Formula I compounds. The drug loading value p is 1 to 8. Formula I compounds include all mixtures of variously loaded and attached antibody-drug conjugates where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody.

In another embodiment, Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36), i.e. not to an ErbB receptor, including HER2.

ANTIBODIES

The antibody unit (Ab-) of Formula I includes within its scope any unit of an antibody that binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population. An antibody can be any protein or protein-like molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. In one aspect, the antibody unit acts to deliver the maytansinoid drug moiety to the particular target cell population with which the antibody unit reacts. Such antibodies include, but are not limited to, large molecular weight proteins such as, full-length antibodies and antibody fragments.

Antibodies comprising the antibody-drug conjugates of the invention preferably retain the antigen binding capability of their native, wild type counterparts. Thus, antibodies of the invention 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, 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). An antigen to which an antibody of the invention is capable of binding may be a member of a subset of one of the above-mentioned categories, wherein the other subset(s) of said category comprise other molecules/antigens that have a distinct characteristic (with respect to the antigen of interest).

In one embodiment, the antibody of the antibody-drug conjugates (ADC) specifically binds to a receptor encoded by an ErbB gene. The antibody may bind specifically to an ErbB receptor selected from EGFR, HER2, HER3 and HER4. The ADC may specifically bind to the extracellular domain (ECD) of the HER2 receptor and inhibit the growth of tumor cells which overexpress HER2 receptor. The antibody of the ADC may be a monoclonal antibody, e.g. a murine monoclonal antibody, a chimeric antibody, or a humanized antibody. A humanized antibody may be huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 or huMAb4D5-8 (trastuzumab). The antibody may be an antibody fragment, e.g. a Fab fragment.

Antibodies in Formula I antibody-drug conjugates (ADC) and which may be useful in the treatment of cancer include, but are not limited to, antibodies against cell surface receptors and tumor-associated antigens (TAA). Such tumor-associated antigens are known in the art, and can prepared for use in generating antibodies using methods and information which are well known in the art. In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Examples of TAA include, but are not limited to, Tumor-Associated Antigens (1)-(36) listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s), following nucleic acid and protein sequence identification conventions of the National Center for Biotechnology Information (NCBI). Nucleic acid and protein sequences corresponding to TAA (1)-(36) are available in public databases such as GenBank. Tumor-associated antigens targeted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the cited references, or which exhibit substantially the same biological properties or characteristics as a TAA having a sequence found in the cited references. For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure in the reference specifically recited herein are expressly incorporated by reference.

TUMOR-ASSOCIATED ANTIGENS (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203) ten Dijke,P., et al Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Claim 2); WO2003042661 (Claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (Claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (Claim 6); WO2003024392 (Claim 2; Fig 112); WO200298358 (Claim 1; Page 183); WO200254940 (Page 100-101); WO200259377(Page 349-350); WO200230268 (Claim 27; Page 376); WO200148204 (Example; Fig 4)

NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1 -

Cross-references: MIM:603248; NP_001194.1; AY065994

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486)
Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H.W., et al (1992) J. Biol. Chem. 267 (16):11267-11273); WO2004048938 (Example 2); WO2004032842 (Example IV); WO2003042661 (Claim 12); WO2003016475 (Claim 1); WO200278524 (Example 2); WO200299074 (Claim 19; Page 127-129); WO200286443 (Claim 27; Pages 222, 393); WO2003003906 (Claim 10; Page 293); WO200264798 (Claim 33; Page 93-95); WO200014228 (Claim 5; Page 133-136); US2003224454 (Fig 3); WO2003025138 (Claim 12; Page 150); US 20050107595; US 20050106644;
NP_003477 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 /pid=NP_003477.3 - Homo sapiens
Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449)
Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., et al (1999) Proc. Natl. Acad. Sci. U.S.A. 96 (25):14523-14528); WO2004065577 (Claim 6); WO2004027049 (Fig 1L); EP1394274 (Example 11); WO2004016225 (Claim 2); WO2003042661 (Claim 12); US2003157089 (Example 5); US2003185830 (Example 5); US2003064397 (Fig 2); WO200289747 (Example 5; Page 618-619); WO2003022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A);
NP_036581 six transmembrane epithelial antigen of the prostate
Cross-references: MIM:604415; NP_036581.1; NM_012449_1

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486)
J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Claim 14); WO200292836 (Claim 6; Fig 12); WO200283866 (Claim 15; Page 116-121); US2003124140 (Example 16); US2003091580 (Claim 6); WO200206317 (Claim 6; Page 400-408);
Cross-references: GI:34501467; AAK74120.3; AF361486_1

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823)
Yamaguchi, N., et al Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. U.S.A. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. U.S.A. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995)); WO2003101283 (Claim 14); (WO2002102235 (Claim 13; Page 287-288); WO2002101075 (Claim 4; Page 308-309); WO200271928 (Page 320-321); WO9410312 (Page 52-57);
Cross-references: MIM:601051; NP_005814.2; NM_005823_1

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b,Genbank accession no. NM_006424)
J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., et al (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582); WO2004022778 (Claim 2); EP1394274 (Example 11);

WO2002102235 (Claim 13; Page 326); EP875569 (Claim 1; Page 17-19); WO200157188 (Claim 20; Page 329); WO2004032842 (Example IV); WO200175177 (Claim 24; Page 139-140);
Cross-references: MIM:604217; NP_006415.1; NM_006424_1

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878)
Nagase T., et al (2000) DNA Res. 7 (2):143-150); WO2004000997 (Claim 1); WO2003003984 (Claim 1); WO200206339 (Claim 1; Page 50); WO200188133 (Claim 1; Page 41-43, 48-58); WO2003054152 (Claim 20); WO2003101400 (Claim 11);
Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628); Ross et al (2002) Cancer Res. 62:2546-2553; US2003129192 (Claim 2); US2004044180 (Claim 12); US2004044179 (Claim 11); US2003096961 (Claim 11); US2003232056 (Example 5); WO2003105758 (Claim 12); US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20);
Cross-references: GI:37182378; AAQ88991.1; AY358628_1

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
Nakamuta M., et al Biochem. Biophys. Res. Commun. 177, 34-39, 1991; Ogawa Y., et al Biochem. Biophys. Res. Commun. 178, 248-255, 1991; Arai H., et al Jpn. Circ. J. 56, 1303-1307, 1992; Arai H., et al J. Biol. Chem. 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., et al Biochem. Biophys. Res. Commun. 178, 656-663, 1991; Elshourbagy N.A., et al J. Biol. Chem. 268, 3873-3879, 1993; Haendler B., et al J. Cardiovasc. Pharmacol. 20, s1-S4, 1992; Tsutsumi M., et al Gene 228, 43-49, 1999; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; Bourgeois C., et al J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., et al Biol. Chem. 272, 21589-21596, 1997; Verheij J.B., et al Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R.M.W., et al Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E.G., et al Cell 79, 1257-1266, 1994; Attie T., et al, Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., et al Hum. Mol. Genet. 5:351-354, 1996; Amiel J., et al Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R.M.W., et al Nat. Genet. 12, 445-447, 1996; Svensson P.J., et al Hum. Genet. 103, 145-148, 1998; Fuchs S., et al Mol. Med. 7, 115-124, 2001; Pingault V., et al (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1); WO2004048938 (Example 2); WO2004040000 (Claim 151); WO2003087768 (Claim 1); WO2003016475 (Claim 1); WO2003016475 (Claim 1); WO200261087 (Fig 1); WO2003016494 (Fig 6); WO2003025138 (Claim 12; Page 144); WO200198351 (Claim 1; Page 124-125); EP522868 (Claim 8; Fig 2); WO200177172 (Claim 1; Page 297-299); US2003109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004001004;

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

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WO2003104275 (Claim 1); WO2004046342 (Example 2); WO2003042661 (Claim 12); WO2003083074 (Claim 14; Page 61); WO2003018621 (Claim 1); WO2003024392 (Claim 2; Fig 93); WO200166689 (Example 6);
Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138)
Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (Claim 1; Fig 1); WO200272596 (Claim 13; Page 54-55); WO200172962 (Claim 1; Fig 4B); WO2003104270 (Claim 11); WO2003104270 (Claim 16); US2004005598 (Claim 22); WO2003042661 (Claim 12); US2003060612 (Claim 12; Fig 10); WO200226822 (Claim 23; Fig 2); WO200216429 (Claim 12; Fig 10);
Cross-references: GI:22655488; AAN04080.1; AF455138_1

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636)
Xu, X.Z., et al Proc. Natl. Acad. Sci. U.S.A. 98 (19):10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33):30813-30820 (2003)); US2003143557 (Claim 4); WO200040614 (Claim 14; Page 100-103); WO200210382 (Claim 1; Fig 9A); WO2003042661 (Claim 12); WO200230268 (Claim 27; Page 391); US2003219806 (Claim 4); WO200162794 (Claim 14; Fig 1A-D);
Cross-references: MIM:606936; NP_060106.2; NM_017636_1

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212)
Ciccodicola, A., et al EMBO J. 8 (7):1987-1991 (1989), Am. J. Hum. Genet. 49 (3):555-565 (1991)); US2003224411 (Claim 1); WO2003083041 (Example 1); WO2003034984 (Claim 12); WO200288170 (Claim 2; Page 52-53); WO2003024392 (Claim 2; Fig 58); WO200216413 (Claim 1; Page 94-95, 105); WO200222808 (Claim 2; Fig 1); US5854399 (Example 2; Col 17-18); US5792616 (Fig 2);
Cross-references: MIM:187395; NP_003203.1; NM_003212_1

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004)
Fujisaku et al (1989) J. Biol. Chem. 264 (4):2118-2125); Weis J.J., et al J. Exp. Med. 167, 1047-1066, 1988; Moore M., et al Proc. Natl. Acad. Sci. U.S.A. 84, 9194-9198, 1987; Barel M., et al Mol. Immunol. 35, 1025-1031, 1998; Weis J.J., et al Proc. Natl. Acad. Sci. U.S.A. 83, 5639-5643, 1986; Sinha S.K., et al (1993) J. Immunol. 150, 5311-5320; WO2004045520 (Example 4); US2004005538 (Example 1); WO2003062401 (Claim 9); WO2004045520 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Claim 1);
Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626 or 11038674)
Proc. Natl. Acad. Sci. U.S.A. (2003) 100 (7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller et al (1992) Eur. J. Immunol. 22 (6):1621-1625); WO2004016225 (claim 2, Fig 140); WO2003087768, US2004101874 (claim 1, page 102); WO2003062401 (claim 9); WO200278524 (Example 2); US2002150573 (claim 5, page 15); US5644033; WO2003048202 (claim 1, pages 306 and 309); WO 99/558658, US6534482 (claim 13, Fig 17A/B); WO200055351 (claim 11, pages 1145-1146);
Cross-references: MIM:147245; NP_000617.1; NM_000626_1

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764, AY358130)
Genome Res. 13 (10):2265-2270 (2003), Immunogenetics 54 (2):87-95 (2002), Blood 99 (8):2662-2669 (2002), Proc. Natl. Acad. Sci. U.S.A. 98 (17):9772-9777 (2001), Xu, M.J., et al (2001) Biochem. Biophys. Res. Commun. 280 (3):768-775; WO2004016225 (Claim 2); WO2003077836; WO200138490 (Claim 5; Fig 18D-1-18D-2); WO2003097803 (Claim 12); WO2003089624 (Claim 25);
Cross-references: MIM:606509; NP_110391.2; NM_030764_1

(17) HER2 (ErbB2, Genbank accession no. M11730)
Coussens L., et al Science (1985) 230(4730):1132-1139); Yamamoto T., et al Nature 319, 230-234, 1986; Semba K., et al Proc. Natl. Acad. Sci. U.S.A. 82, 6497-6501, 1985; Swiercz J.M., et al J. Cell Biol. 165, 869-880, 2004; Kuhns J.J., et al J. Biol. Chem. 274, 36422-36427, 1999; Cho H.-S., et al Nature 421, 756-760, 2003; Ehsani A., et al (1993) Genomics 15, 426-429; WO2004048938 (Example 2); WO2004027049 (Fig 1I); WO2004009622; WO2003081210; WO2003089904 (Claim 9); WO2003016475 (Claim 1); US2003118592; WO2003008537 (Claim 1); WO2003055439 (Claim 29; Fig 1A-B); WO2003025228 (Claim 37; Fig 5C); WO200222636 (Example 13; Page 95-107); WO200212341 (Claim 68; Fig 7); WO200213847 (Page 71-74); WO200214503 (Page 114-117); WO200153463 (Claim 2; Page 41-46); WO200141787 (Page 15); WO200044899 (Claim 52; Fig 7); WO200020579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4);
Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

(18) NCA (CEACAM6, Genbank accession no. M18728);
Barnett T., et al Genomics 3, 59-66, 1988; Tawaragi Y., et al Biochem. Biophys. Res. Commun. 150, 89-96, 1988; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903, 2002; WO2004063709; EP1439393 (Claim 7); WO2004044178 (Example 4); WO2004031238; WO2003042661 (Claim 12); WO200278524 (Example 2); WO200286443 (Claim 27; Page 427); WO200260317 (Claim 2);
Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;

(19) MDP (DPEP1, Genbank accession no. BC017023)

364 of

Proc. Natl. Acad. Sci. U.S.A. 99 (26):16899-16903 (2002)); WO2003016475 (Claim 1); WO200264798 (Claim 33; Page 85-87); JP05003790 (Fig 6-8); WO9946284 (Fig 9); Cross-references: MIM:179780; AAH17023.1; BC017023_1

(20) IL20Rα (IL20Ra, ZCYTOR7, Genbank accession no. AF184971); Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Mungall A.J., et al Nature 425, 805-811, 2003; Blumberg H., et al Cell 104, 9-19, 2001; Dumoutier L., et al J. Immunol. 167, 3545-3549, 2001; Parrish-Novak J., et al J. Biol. Chem. 277, 47517-47523, 2002; Pletnev S., et al (2003) Biochemistry 42:12617-12624; Sheikh F., et al (2004) J. Immunol. 172, 2006-2010; EP1394274 (Example 11); US2004005320 (Example 5); WO2003029262 (Page 74-75); WO2003002717 (Claim 2; Page 63); WO200222153 (Page 45-47); US2002042366 (Page 20-21); WO200146261 (Page 57-59); WO200146232 (Page 63-65); WO9837193 (Claim 1; Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) Brevican (BCAN, BEHAB, Genbank accession no. AF229053) Gary S.C., et al Gene 256, 139-147, 2000; Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; US2003186372 (Claim 11); US2003186373 (Claim 11); US2003119131 (Claim 1; Fig 52); US2003119122 (Claim 1; Fig 52); US2003119126 (Claim 1); US2003119121 (Claim 1; Fig 52); US2003119129 (Claim 1); US2003119130 (Claim 1); US2003119128 (Claim 1; Fig 52); US2003119125 (Claim 1); WO2003016475 (Claim 1); WO200202634 (Claim 1);

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442) Chan,J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000)); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42); Cross-references: MIM:600997; NP_004433.2; NM_004442_1

(23) ASLG659 (B7h, Genbank accession no. AX092328) US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); US2003165504 (Claim 1); US2003124140 (Example 2); US2003065143 (Fig 60); WO2002102235 (Claim 13; Page 299); US2003091580 (Example 2); WO200210187 (Claim 6; Fig 10); WO200194641 (Claim 12; Fig 7b); WO200202624 (Claim 13; Fig 1A-1B); US2002034749 (Claim 54; Page 45-46); WO200206317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587 (Example 1; Fig 1); WO200140269 (Example 3; Pages 190-192); WO200036107 (Example 2; Page 205-207); WO2004053079 (Claim 12); WO2003004989 (Claim 1); WO200271928 (Page 233-234, 452-453); WO 0116318;

(24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436)

Reiter R.E., et al Proc. Natl. Acad. Sci. U.S.A. 95, 1735-1740, 1998; Gu Z., et al Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-788; WO2004022709; EP1394274 (Example 11); US2004018553 (Claim 17); WO2003008537 (Claim 1); WO200281646 (Claim 1; Page 164); WO2003003906 (Claim 10; Page 288); WO200140309 (Example 1; Fig 17); US2001055751 (Example 1; Fig 1b); WO200032752 (Claim 18; Fig 1); WO9851805 (Claim 17; Page 97); WO9851824 (Claim 10; Page 94); WO9840403 (Claim 2; Fig 1B); Accession: O43653; EMBL; AF043498; AAC39607.1.

(25) GEDA (Genbank accession No. AY260763); AAP14954 lipoma HMGIC fusion-partner-like protein /pid=AAP14954.1 - Homo sapiens Species: Homo sapiens (human) WO2003054152 (Claim 20); WO2003000842 (Claim 1); WO2003023013 (Example 3, Claim 20); US2003194704 (Claim 45); Cross-references: GI:30102449; AAP14954.1; AY260763_1

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, Genbank accession No. AF116456); BAFF receptor /pid=NP_443177.1 - Homo sapiens Thompson, J.S., et al Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611; WO2003045422 (Example; Page 32-33); WO2003014294 (Claim 35; Fig 6B); WO2003035846 (Claim 70; Page 615-616); WO200294852 (Col 136-137); WO200238766 (Claim 3; Page 133); WO200224909 (Example 3; Fig 3); Cross-references: MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814, Genbank accession No. AK026467); Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Claim 1; Fig 1); Cross-references: MIM:107266; NP_001762.1; NM_001771_1

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation) PROTEIN SEQUENCE Full mpgggpgv...dvqlekp (1..226; 226 aa), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.10) WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4, pages 13-14); WO9958658 (claim 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha et al (1992) J. Immunol. 148(5):1526-1531; Mueller et al (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et al (1994) Immunogenetics 40(4):287-295; Preud'homme et al (1992) Clin. Exp. Immunol. 90(1):141-146; Yu et al (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al (1988) EMBO J. 7(11):3457-3464;

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and

perhaps development of AIDS, lymphoma, myeloma, and leukemia) PROTEIN SEQUENCE Full mnypltl...atsltf (1..372; 372 aa), pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No. NP_001707.1)
WO2004040000; WO2004015426; US2003105292 (Example 2); US6555339 (Example 2); WO200261087 (Fig 1); WO200157188 (Claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7, Fig 5); Dobner et al (1992) Eur. J. Immunol. 22:2795-2799; Barella et al (1995) Biochem. J. 309:773-779;

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes) PROTEIN SEQUENCE Full mgsgwvp...vllpqsc (1..273; 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1)
Tonnelle et al (1985) EMBO J. 4(11):2839-2847; Jonsson et al (1989) Immunogenetics 29(6):411-413; Beck et al (1992) J. Mol. Biol. 228:433-441; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al (1987) J. Biol. Chem. 262:8759-8766; Beck et al (1996) J. Mol. Biol. 255:1-13; Naruse et al (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-146); Kasahara et al (1989) Immunogenetics 30(1):66-68; Larhammar et al (1985) J. Biol. Chem. 260(26):14111-14119;

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability) PROTEIN SEQUENCE Full mgqagck...lephrst (1..422; 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2)
Le et al (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman et al (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, Genbank accession No. NP_001773.1)
WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-106); Von Hoegen et al (1990) J. Immunol. 144(12):4870-4877; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis) PROTEIN SEQUENCE Full mafdvsc...rwkyqhi (1..661; 661 aa), pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1)
US2002193567; WO9707198 (claim 11, pages 39-42); Miura et al (1996) Genomics 38(3):299-304; Miura et al (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-61); WO200012130 (pages 24-26);

(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation) PROTEIN SEQUENCE Full mlprlll...vdyedam (1..429; 429 aa), pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, Genbank accession No. NP_443170.1)
WO2003077836; WO200138490 (claim 6, Fig 18E-1-18-E-2); Davis et al (2001) Proc. Natl. Acad. Sci USA 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1); WO2003089624 (claim 7);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies) PROTEIN SEQUENCE Full mllwvil...assaphr (1..977; 977 aa), pI: 6.88 MW: 106468 TM: 1 [P] Gene Chromosome: 1q21, Genbank accession No. Human:AF343662, AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse:AK089756, AY158090, AY506558; NP_112571.1
WO2003024392 (claim 2, Fig 97); Nakayama et al (2000) Biochem. Biophys. Res. Commun. 277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2);

(36) TENB2 (TMEFF2, tomoregulin, TPEF, HPP1, TR, putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin) PROTEIN SEQUENCE Full mvlwesp...rastrli (1..374; 374 aa, NCBI Accession: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; Genbank accession No. AF179274; AY358907, CAF85723, CQ782436
WO2004074320 (SEQ ID NO 810); JP2004113151 (SEQ ID NOS 2, 4, 8); WO2003042661 (SEQ ID NO 580); WO2003009814 (SEQ ID NO 411); EP1295944 (pages 69-70); WO200230268 (page 329); WO200190304 (SEQ ID NO 2706); US2004249130; US2004022727; WO2004063355; US2004197325; US2003232350; US2004005563; US2003124579; US 6410506; US 6642006l; Horie et al (2000) Genomics 67:146-152; Uchida et al (1999) Biochem. Biophys. Res. Commun. 266:593-602; Liang et al (2000) Cancer Res. 60:4907-12; Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84.

PRODUCTION OF ANTIBODIES

Various methods have been employed to produce monoclonal antibodies (MAbs). Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Other methods to prepare MAbs, including chimeric and humanized antibodies, uses genetic engineering, i.e. recombinant DNA techniques.

Polyclonal antibodies may be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts.

Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, (1984) J. Immunol., 133:3001, and Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York,

1987)). Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al (1980) Anal. Biochem. 107:220.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells (US 2005/0048572; US 2004/0229310). Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al (1993) Curr. Opinion in Immunol. 5:256-262 and Plückthun (1992) Immunol. Revs. 130:151-188.

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

The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (US 4816567); and Morrison et al (1984) Proc. Natl Acad. Sci. USA 81:6851), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

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

A description follows as to exemplary techniques for the production of the antibodies (Ab) used in the antibody-drug conjugates (ADC) of the present invention. The production of antibodies will be illustrated with reference to anti-ErbB2 antibodies but it will be apparent for those skilled in the art that antibodies to other members of the ErbB receptor family, as well as any other receptor or tumor-associated antigen or target, can be produced and modified in a similar manner.

The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface, e.g. NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma

cell line such as SK-BR-3 cells (Stancovski et al (1991) PNAS (USA) 88:8691-8695), can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

Example 1 describes production of an exemplary humanized anti-ErbB2 antibody. The humanized antibody may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth 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, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H.

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production (Jakobovits et al (1993) Proc. Natl. Acad. Sci. USA, 90:2551; Jakobovits et al (1993) Nature 362:255-258; Bruggermann et al (1993) Year in Immuno. 7:33; and US 5591669; US 5589369; US 5545807).

Alternatively, phage display technology (McCafferty et al (1990) Nature 348:552-553) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors (Johnson, Kevin S. and Chiswell, David J. (1993) *Current Opinion in Structural Biology* 3:564-571). A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially (Marks et al (1991) J. Mol. Biol. 222:581-597; Griffith et al (1993) EMBO J. 12:725-734; US 5565332; US 5573905). Human antibodies may also be generated by *in vitro* activated B cells (US 5567610; US 5229275). Human anti-ErbB2 antibodies are described (US 5772997 and WO 97/00271.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see Morimoto et al (1992) Journal of Biochemical and Biophysical Methods 24:107-117; and Brennan et al (1985) Science 229:81). Antibody fragments can also be produced directly by recombinant host cells and the antibody phage libraries discussed above. 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). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; US 5571894; and US 5587458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US 5641870 for example. Such linear antibody fragments may be monospecific or bispecific.

Bispecific antibodies with binding specificities for at least two different epitopes (Millstein et al (1983), Nature 305:537-539) may bind to two different epitopes of the ErbB2 protein. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2 (WO 96/16673; US

5837234; WO98/02463; US 5821337). Purification methods for bispecific antibodies have been disclosed (WO 93/08829; Traunecker et al (1991) EMBO J. 10:3655-3659; WO 94/04690; Suresh et al (1986) *Methods in Enzymology* 121:210; US 5731168). Bispecific antibodies can be produced using leucine zippers (Kostelny et al (1992) J. Immunol. 148(5):1547-1553), and single-chain Fv (sFv) dimers (Gruber et al (1994) J. Immunol. 152:5368).

Techniques for generating bispecific antibodies from antibody fragments have also been described, such as using chemical linkage wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments (Brennan et al (1985) Science 229:81). Fab'-SH fragments can be recovered from *E. coli* and chemically coupled to form bispecific antibodies (Shalaby et al (1992) J. Exp. Med. 175:217-225. The "diabody" technology provides an alternative method for making bispecific antibody fragments (Hollinger et al (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448).

Antibodies with more than two valencies are contemplated. Multivalent, "Octopus" antibodies with three or more antigen binding sites and two or more variable domains can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody (US 2002/0004586; WO 01/77342). For example, trispecific antibodies can be prepared (Tutt et al (1991) J. Immunol. 147:60.

Amino acid sequence modification(s) of antibodies are contemplated. For example, mutants and various isoforms of antibodies which bind to tumor-associated antigens are contemplated to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody are prepared by introducing appropriate nucleotide changes into the nucleic acid 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 is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is "alanine scanning mutagenesis" (Cunningham and Wells (1989) Science 244:1081-1085) where an amino acid 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, such as alanine or polyalanine, to optimize the interaction of the amino acids with antigen. 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 anti-ErbB2 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-ErbB2 antibody molecule include the fusion to the N- or C-terminus of the anti-ErbB2 antibody to an enzyme (e.g. for ADEPT: Tietze et al (2003) Current Pharm. Design 9:2155-2175) or a polypeptide which increases the serum half-life of the antibody, such as an albumin-binding peptide.

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

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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, SEQ ID NOS: z1-z14, and all of which are incorporated herein by reference.

The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. 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. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

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. Naturally occurring residues are divided into groups based on common side-chain properties:

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

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

(3) acidic: asp, glu;

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

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

(6) aromatic: trp, tyr, phe.

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

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

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., IgG_1, IgG_2, IgG_3, or IgG_4) 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 5834597).

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), or the extent of glycosylation.

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Antibodies may be glycosylated at conserved positions (N-linked or O-linked) in their constant regions (Hse et al (1997) J. Biol. Chem. 272:9062-9070; Jefferis and Lund, (1997) Chem. Immunol. 65:111-128; Wright and Morrison, (1997) TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al (1996) Mol. Immunol. 32:1311-1318; Wittwe and Howard, (1990) Biochem. 29:4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner (1996) Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures (Malhotra et al (1995) Nature Med. 1:237-243; Umana et al (1999) Nature Biotech. 17:176-180). Removal of the oligosaccharides may optimize antigen binding and other properties of the antibody (Boyd et al (1996) Mol. Immunol. 32:1311-1318).

Factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like (US 5047335; US 5510261; US 5278299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo-β-galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

MAYTANSINOID DRUG MOIETIES

Maytansine compounds inhibit cell proliferation by inhibiting the formation of microtubules during mitosis through inhibition of polymerization of the microtubulin protein, tubulin (Remillard et al (1975) Science 189:1002-1005; US 5208020). Maytansine and maytansinoids are highly cytotoxic but their clinical use in cancer therapy has been greatly limited by their severe systemic side-effects primarily attributed to their poor selectivity for tumors. Clinical trials with maytansine had been discontinued due to serious adverse effects on the central nervous system and gastrointestinal system (Issel et al (1978) Can. Treatment. Rev. 5:199-207.

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.

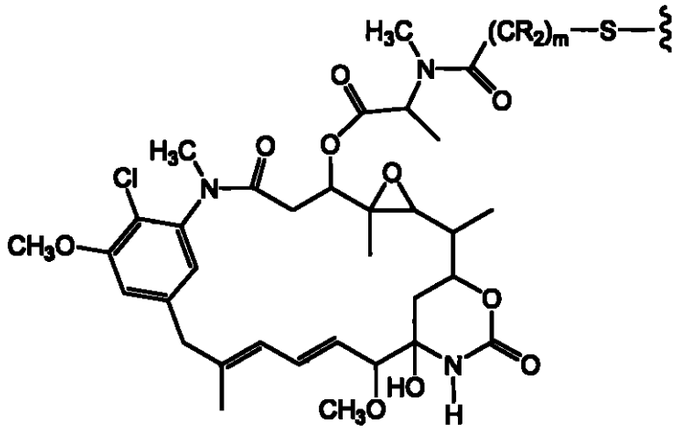
Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared 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 ansamitocin 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 (US 4294757) (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, prepared by the reaction of maytansinol with H_2S or P_2S_5 (US 4424219); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) prepared from *Nocardia* (US 4450254); C-15-hydroxy/acyloxy, prepared by the conversion of maytansinol by Streptomyces (US 4364866); C-15-methoxy, isolated from Trewia nudiflora (US 4313946 and US 4315929); C-18-N-demethyl, prepared by the demethylation of maytansinol by Streptomyces (US 4362663 and US 4322348); and 4,5-deoxy, prepared by the titanium trichloride/LAH reduction of maytansinol (US 4371533).

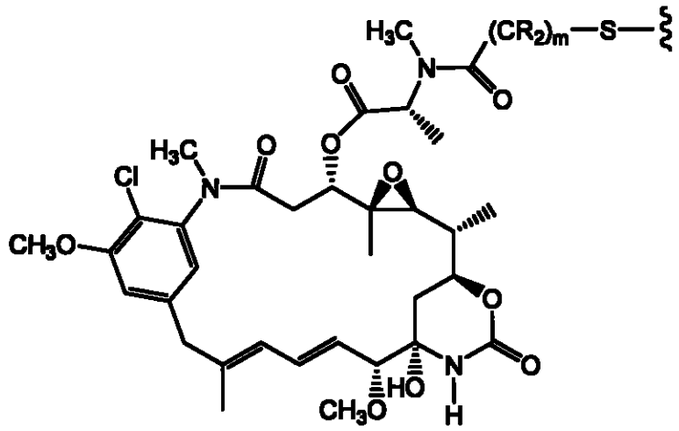
Many positions on maytansine compounds are known to be useful as the linkage position, depending upon the type of link. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group and the C-20 position having a hydroxyl group are all suitable.

Maytansinoid drug moieties (D) include those having the structure:



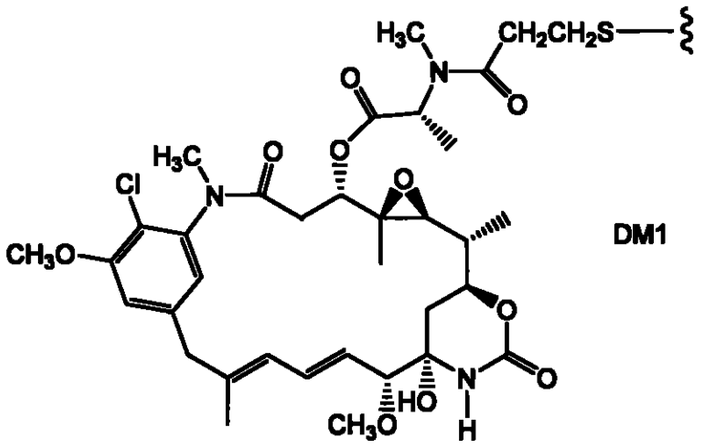
where the wavy line indicates the covalent attachment of the sulfur atom of D to a linker (L) of an antibody-drug conjugate (ADC). R may independently be H or a C_1–C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e. m is 1, 2, or 3 (US 633410, 5208020, Chari et al (1992) Cancer Res. 52:127-131; Liu et al (1996) Proc. Natl. Acad. Sci 93:8618-8623).

All stereoisomers of the maytansinoid drug moiety are contemplated for the compounds of the invention, i.e. any combination of *R* and *S* configurations at the chiral carbons of D. In one embodiment, the maytansinoid drug moiety (D) will have the following stereochemistry:



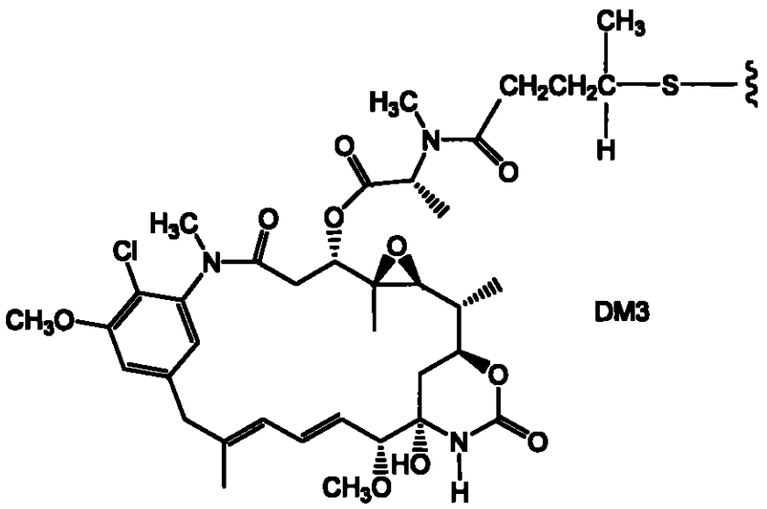
Embodiments of D include:

DM1 ($N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine) where $(CR_2)_m = CH_2CH_2$;



DM1

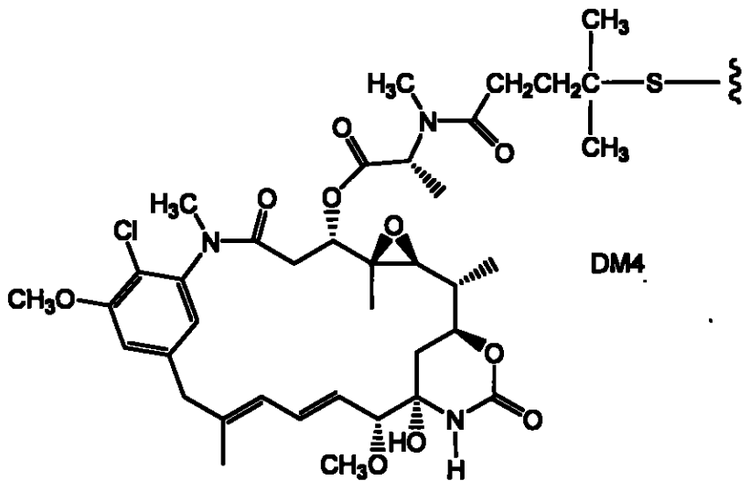
DM3 ($N^{2'}$-deacetyl-N-$^{2'}$(4-mercapto-1-oxopentyl)-maytansine) where $(CR_2)_m = CH_2CH_2CH(CH_3)$;



DM3

DM4 ($N^{2'}$-deacetyl-$N^{2'}$-(4-methyl-4-mercapto-1-oxopentyl)-maytansine) where $(CR_2)_m$ =

$CH_2CH_2C(CH_3)_2$:



DM4

Steric hindrance conferred by alkyl groups such as the methyl groups on the carbon adjacent to the sulfur atom of DM3 and DM4 may affect the rate of intracellular cleavage of the ADC (US 2004/0235840 A1). The variable alkyl unit $(CR_2)_m$ may therefore affect potency, efficacy, and safety/toxicity in vitro and in vivo.

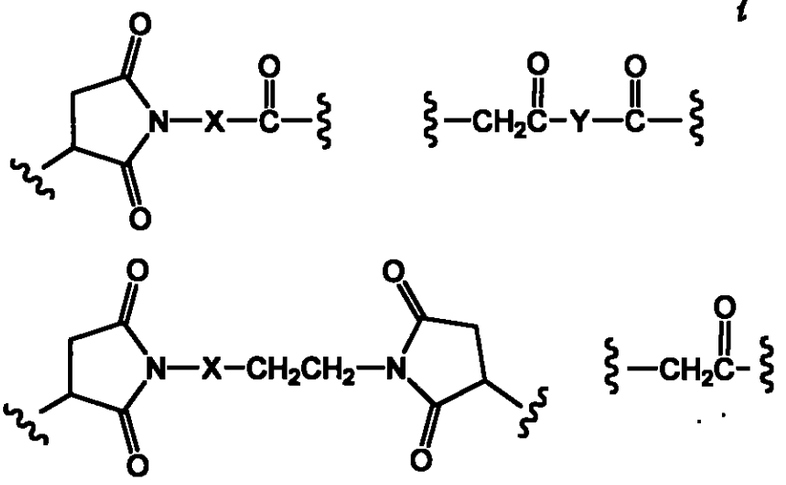
LINKERS

The linker, L, attaches the antibody to a drug moiety through covalent bond(s), not comprising a disulfide group. The linker 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, or an amine, e.g. N-terminus or amino acid side chain such as lysine, of the antibody (Ab) can form a bond with a functional group of a linker reagent, drug moiety or drug-linker reagent.

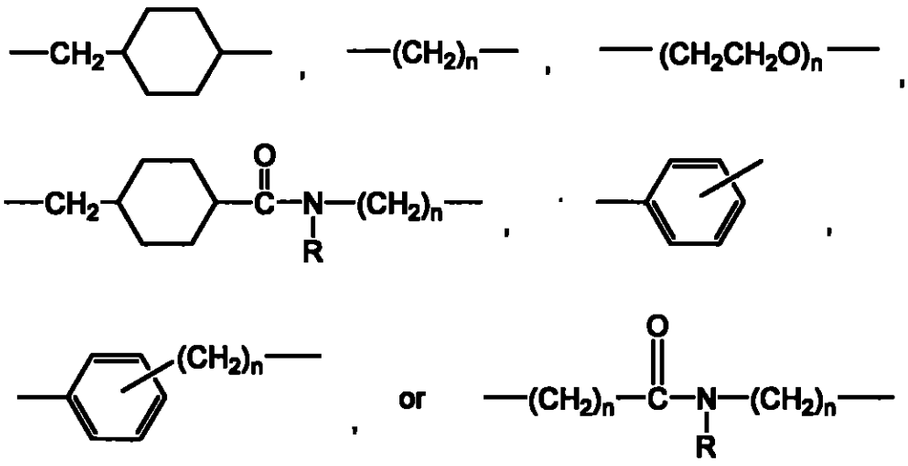
The linkers are preferably stable extracellularly. Before transport or delivery into a cell, the antibody-drug conjugate (ADC) is preferably stable and remains intact, i.e. the antibody remains linked to the drug moiety. The linkers are stable outside the target cell and may be cleaved at some efficacious rate inside the cell. An effective linker will: (i) maintain the specific binding properties of the antibody; (ii) allow intracellular delivery of the conjugate or drug moiety; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its targetted site; and (iv) maintain a cytotoxic, cell-killing effect or a cytostatic effect of the maytansinoid drug moiety. Stability of the ADC may be measured by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS.

Covalent attachment of the antibody and the drug moiety requires the linker to have two reactive functional groups, i.e. bivalency in a reactive sense. Bivalent linker reagents which are useful to attach two or more functional or biologically active moieties, such as peptides, nucleic acids, drugs, toxins, antibodies, haptens, and reporter groups are known, and methods have been described their resulting conjugates (Hermanson, G.T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p234-242).

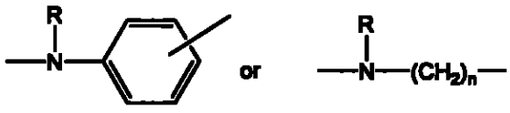
Linkers may have structures selected from:



where the wavy lines indicate the covalent attachments to Ab and D in either orientation. X may have the structures, in either orientation:

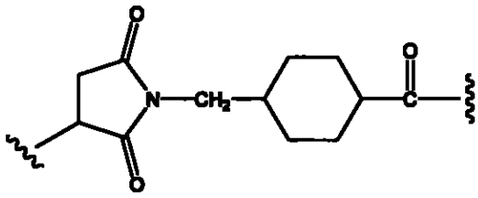


where R is independently H or C_1–C_6 alkyl; and n is 1 to 12. Y may have the structures, in either orientation:

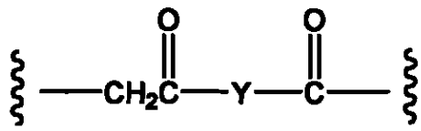


where R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

For example, the linker may have the structure, designated as SMCC:

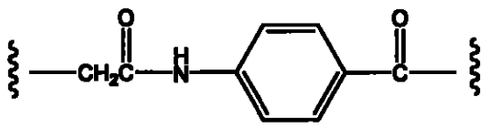


In another embodiment, linker (L) has the structure:

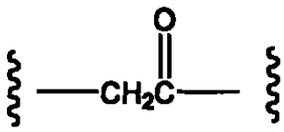


where the wavy lines indicate the covalent attachments to Ab and D in either orientation.

For example, the linker may have the structure, designated as SIAB:



In another embodiment, linker (L) has the structure:



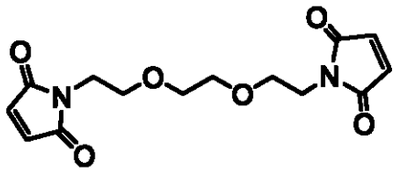
In another embodiment, the linker may be substituted with groups which modulated solubility or reactivity. For example, a sulfonate substituent may increase water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody or the drug moiety, or facilitate the coupling reaction of Ab-L with D, or D-L with Ab, depending on the synthetic route employed to prepare the ADC.

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.

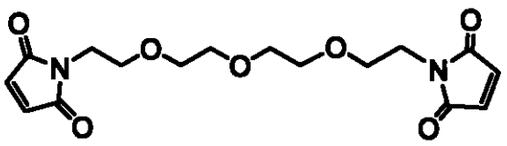
Linkers can be peptidic, comprising one or more amino acid units. Peptide linker reagents may be prepared by solid phase or liquid phase synthesis methods (E. Schröder and K. Lübke, *The Peptides*, volume 1, pp 76-136 (1965) Academic Press) that are well known in the field of peptide chemistry, including t-BOC chemistry (Geiser et al "Automation of solid-phase peptide synthesis" in *Macromolecular Sequencing and Synthesis*, Alan R. Liss, Inc., 1988, pp. 199-218) and Fmoc/HBTU chemistry (Fields, G. and Noble, R. (1990) "Solid phase peptide synthesis utilizing 9-fluoroenylmethoxycarbonyl amino acids", Int. J. Peptide Protein Res. 35:161-214), on an automated synthesizer such as the Rainin Symphony Peptide Synthesizer (Protein Technologies, Inc., Tucson, AZ), or Model 433 (Applied Biosystems, Foster City, CA).

The compounds expressly contemplate, but are not limited to, ADC prepared with cross-linker reagents: BMPEO, 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), 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. See pages 467-498, 2003-2004 Applications Handbook and Catalog. Bis-maleimide reagents allow the attachment of a free thiol group of a cysteine residue of an 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 an antibody, maytansinoid drug moiety, or linker intermediate include iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

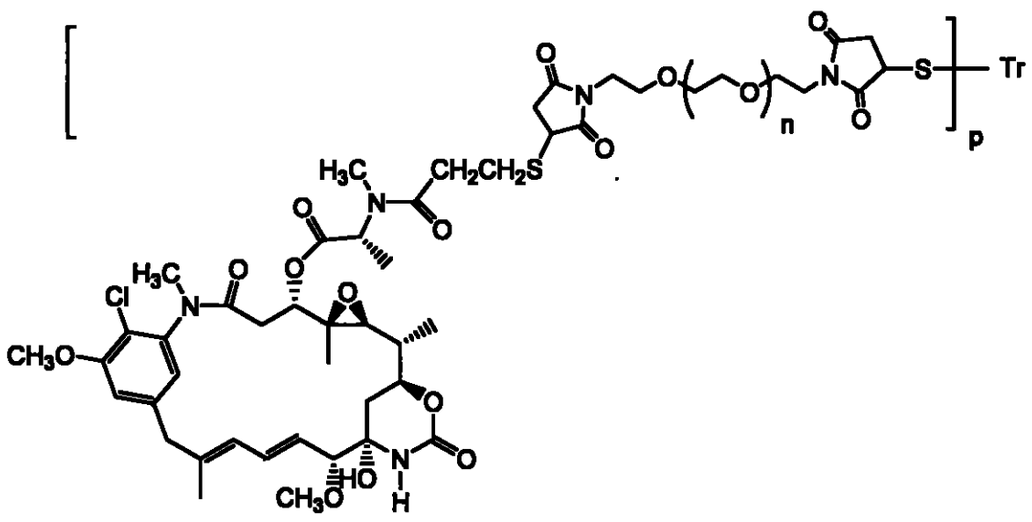


BM(PEO)$_3$



BM(PEO)$_4$

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

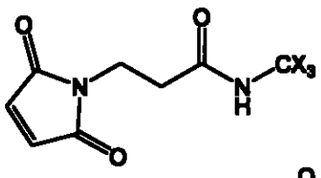


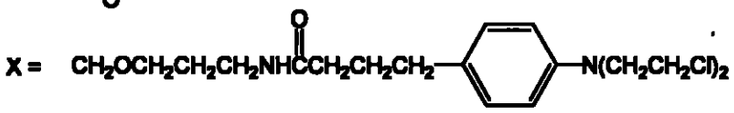
where Tr is trastuzumab; n is 0, 1, or 2; and p is 1, 2, 3, or 4.

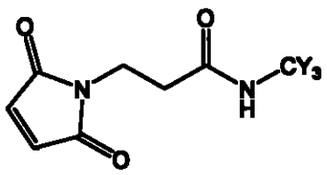
Useful linker reagents can also be obtained via other commercial sources, such as Molecular Biosciences Inc.(Boulder, CO), or synthesized in accordance with procedures described in Toki et al (2002) J. Org. Chem. 67:1866-1872; US 6214345 to Firestone et al; WO 02/088172; US 2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

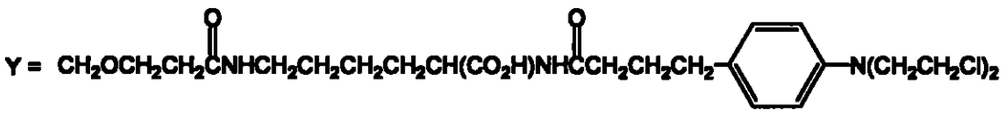
The Linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to an antibody (Sun et al (2002) Bioorganic & Medicinal Chemistry Letters 12:2213-2215; Sun et al (2003) Bioorganic & Medicinal Chemistry 11:1761-1768; King et al (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 an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

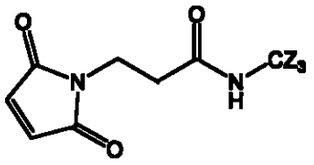
The following exemplary embodiments of dendritic linker reagents allow up to nine nucleophilic drug moiety reagents to be conjugated by reaction with the chloroethyl nitrogen mustard functional groups:

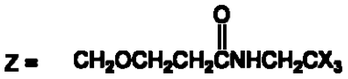












or



DRUG LOADING

The drug loading is represented by p in a molecule of Formula I, the average number of maytansinoid drugs per antibody. Drug loading may range from 1 to 8 drugs (D) per antibody (Ab), i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody. Compositions of ADC of Formula I include collections of antibodies conjugated with a range of drugs, from 1 to 8. The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, electrophoresis, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clinical Cancer Res. 10:7063-7070; Sanderson et al (2005) Clinical Cancer Res. 11:843-852). However, the distribution of p (drug) values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also, ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. 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. Higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the drug-linker intermediate (D-L) or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. Additionally, the antibody must be 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 several different manners, including: (i) limiting the molar excess of drug-linker intermediate (D-L) or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

Where more than one nucleophilic or electrophilic group of the antibody 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 drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (p) may be isolated ("Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate", Hamblett, K.J., et al, Abstract No. 624, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; "Controlling the Location of Drug Attachment in Antibody-Drug Conjugates", Alley, S.C., et al, Abstract No. 627, American Association for Cancer Research; 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). However, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody.

PREPARATION OF 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 nucleophilic group or an electrophilic group of an antibody with a bivalent 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 or an electrophilic group of a drug moiety with a linker reagent, to form drug-linker intermediate D-L, via a covalent bond, followed by reaction with the nucleophilic group or an electrophilic group of an antibody. Conjugation methods (1) and (2) may be employed with a variety of antibodies, drug moieties, and linkers to prepare the antibody-drug conjugates of Formula I.

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 (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). Each cysteine disulfide 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.

Antibody-drug conjugates may also be produced by modification of the antibody to introduce electrophilic moieties, which can react with nucleophilic substituents 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 galactose 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, G.T. (1996) *Bioconjugate Techniques*; Academic Press: New York, p234-242). 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.

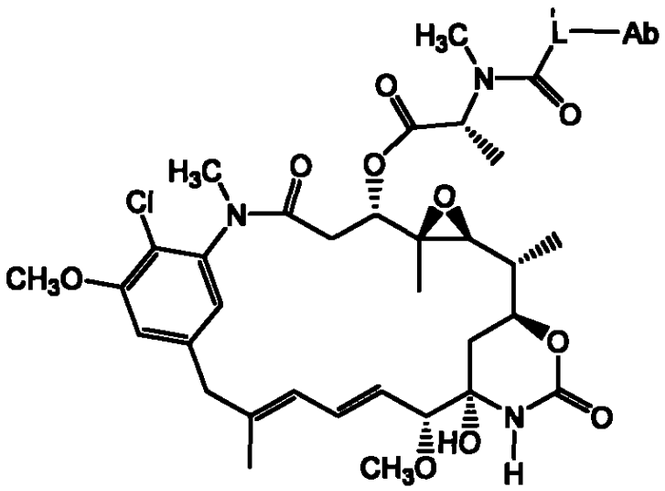
Maytansine may, for example, be converted to May-$SSCH_3$, which can be reduced to the free thiol, May-SH, and reacted with a modified antibody (Chari et al (1992) Cancer Research 52:127-131) to generate a maytansinoid-antibody immunoconjugate with a disulfide linker. Antibody-maytansinoid conjugates with disulfide linkers have been reported (WO 04/016801; US 6884874; US 2004/039176 A1; WO 03/068144; US 2004/001838 A1; US 6441163; US 5208020; US 5416064; WO 01/024763). The disulfide linker SPP is constructed with linker reagent N-succinimidyl 4-(2-pyridylthio) pentanoate. Antibody-SPP-DM1 conjugates are represented by the structure:

Disulfide (S-S) linker antibody-drug conjugates were tested for purposes of comparison to the non-disulfide linker ADC of the invention. Trastuzumab-SPP-DM1 was prepared according to Example 3 (Ranson, M. and Sliwkowski M. (2002) Oncology 63(suppl 1):17-24). Disulfide antibody-drug conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM3, and trastuzumab-SPP-DM4 were also tested, and have the structures

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below:

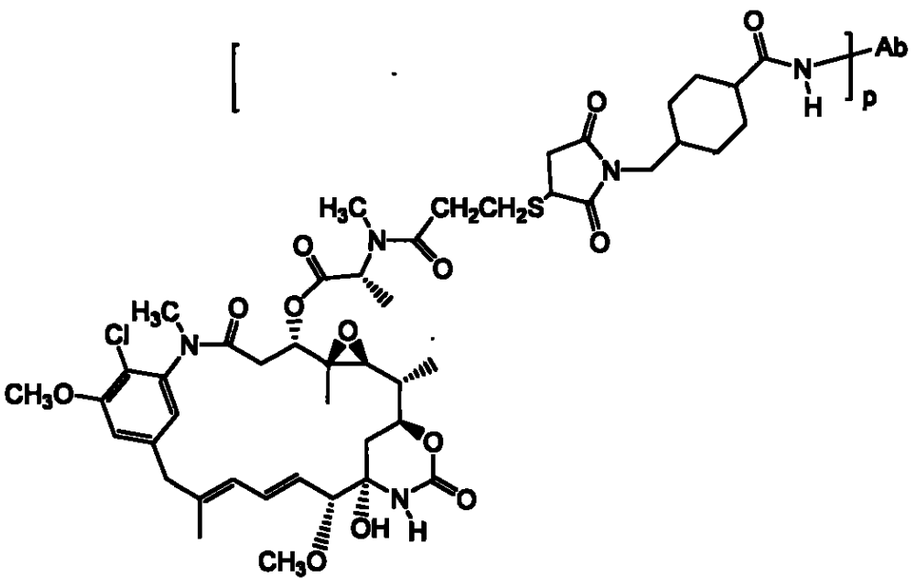


L—Ab = CH_2CH_2-S-S-CH_2CH_2C(=O)NH—Tr Trastuzumab-SPDP-DM1

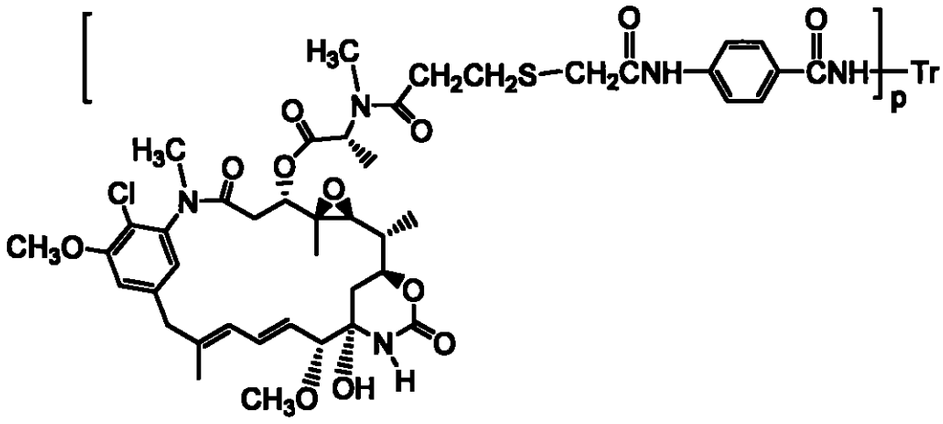
L—Ab = $CH_2CH(CH_3)$-S-S-$CH(CH_3)CH_2CH_2$C(=O)NH—Tr Trastuzumab-SPP-DM3

L—Ab = $CH_2C(CH_3)_2$-S-S-$CH(CH_3)CH_2CH_2$C(=O)NH—Tr Trastuzumab-SPP-DM4

ADC of the invention include SMCC linkers and the DM1 maytansinoid drug moiety, represented as Ab-SMCC-DM1:



One embodiment of Ab-SMCC-DM1 is trastuzumab-SMCC-DM1 where p is 1, 2, 3, or 4 (Ab = trastuzumab, Tr, WO 2005/037992). Another embodiment of an ADC is trastuzumab-SIAB-DM1 (trastuzumab = Tr) having the structure:



SCREENING FOR ANTIBODY-DRUG CONJUGATES (ADC) DIRECTED AGAINST TUMOR-ASSOCIATED ANTIGENS AND CELL SURFACE RECEPTORS

Transgenic animals and cell lines are particularly useful in screening antibody-drug conjugates (ADC) that have potential as prophylactic or therapeutic treatments of diseases or disorders involving overexpression of tumor-associated antigens and cell surface receptors, e.g. HER2 (US 6632979). Screening for a useful ADC may involve administering candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. Candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format. The rate at which ADC may be screened for utility for prophylactic or therapeutic treatments of diseases or disorders is limited only by the rate of synthesis or screening methodology, including detecting/measuring/analysis of data.

One embodiment is a screening method comprising (a) transplanting cells from a stable breast cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the formation of tumors from the transplanted cell line. The invention also concerns a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of a receptor protein comprising (a) contacting cells from a stable breast cancer cell line with a drug candidate and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

One embodiment is a screening method comprising (a) contacting cells from a stable breast cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of HER2. Another embodiment the ability of the ADC candidate to block heregulin binding is evaluated. In another embodiment the ability of the ADC candidate to block ligand-stimulated tyrosine phosphorylation is evaluated.

Another embodiment is a screening method comprising (a) contacting cells from a stable breast cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

Another embodiment is a screening method comprising (a) administering an ADC drug candidate to a transgenic non-human mammal that overexpresses, e.g. in its mammary gland cells, a native human protein, e.g. HER2 or a fragment thereof, wherein such transgenic mammal has stably integrated into its genome a nucleic acid sequence encoding the native human protein or a fragment thereof having the biological activity of the native human protein, operably linked to transcriptional regulatory sequences directing its expression, and develops a tumor, e.g. a mammary tumor, not responding or poorly responding to antibody treatment, e.g. anti-HER2, or to a non-human mammal bearing a tumor transplanted from said transgenic non-human mammal; and (b) evaluating the effect of the ADC candidate on the target disease or disorder. Without limitations, the disease or disorder may be a HER2-overexpressing cancer, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic and bladder cancer. The cancer may be breast cancer which expressed HER2 in at least about 500,000 copies per cell, or at least about 2,000,000 copies per cell. ADC drug candidates may, for example, be evaluated for their ability to induce cell death and/or apoptosis, using assay methods well known in the art and described hereinafter.

In one embodiment, candidate ADC are screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. Administration may be oral, or by suitable injection, depending on the chemical nature of the compound being evaluated. In some cases, it may be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for compounds useful in treating various disorders associated with overexpression of certain tumor-associated antigen proteins or cell surface receptors, e.g. HER2-overexpression, the test compounds are added to the cell culture medium at an appropriate time, and the cellular response to the compound is evaluated over time using the appropriate biochemical and/or histological assays. In some cases, it may be appropriate to apply the compound of interest to the culture medium in conjunction with co-factors that would enhance the efficacy of the compound.

Thus, the present invention provides assays for identifying ADC which specifically target and bind the overexpressed HER2 protein, the presence of which is correlated with abnormal cellular function, and in the pathogenesis of cellular proliferation and/or differentiation of mammary gland that is causally related to the development of breast tumors.

To identify an ADC which blocks ligand activation of an ErbB (e.g. ErbB2) receptor, the ability of the compound to block ErbB ligand binding to cells expressing the ErbB (ErbB2) receptor (e.g. in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells isolated from the transgenic animal overexpressing HER2 and transfected to express another ErbB receptor (with which HER2 forms hetero-oligomer) may be incubated, i.e. culturing, with the ADC and then exposed to labeled ErbB ligand. The ability of the compound to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of heregulin (HRG) binding to breast tumor cell lines, overexpressing HER2 and established from the transgenic non-human mammals (e.g. mice) herein, by the candidate ADC may be

performed using monolayer cultures on ice in a 24-well-plate format. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRGβ1$_{177-224}$ (25,000 cpm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC_{50} value may be calculated for the compound of interest.

Alternatively, or additionally, the ability of an ADC to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cell lines established from the transgenic animals herein may be incubated with a test ADC and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal antibody (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in US 5766863 is also available for determining ErbB receptor activation and blocking of that activity by the compound.

In one embodiment, one may screen for ADC which inhibit HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described below. For example, a cell line established from a HER2-transgenic animal may be plated in 24-well plates and the compound may be added to each well and incubated for 30 minutes at room temperature; then rHRGβ$_{1177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for about 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 µl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 µl) may be electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 µg/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r ~180,000 may be quantified by reflectance densitometry. An alternate method to evaluate inhibition of receptor phosphorylation is the KIRA (kinase receptor activation) assay (Sadick et al (1998) Jour. of Pharm. and Biomed. Anal. 1-9). Some of the well-established monoclonal antibodies against HER2 that are known to inhibit HRG stimulation of p180 tyrosine phosphorylation can be used as positive control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC_{50} for the compound of interest may be calculated.

One may also assess the growth inhibitory effects of a test ADC on cell lines derived from a HER2-transgenic animal (Schaefer et al (1997) Oncogene 15:1385-1394). According to this assay, the cells may be treated with a test compound at various concentrations for 4 days and stained with crystal violet or the redox dye Alamar Blue. Incubation with the compound may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4 on MDA-MB-175 cells (Schaefer et al., supra). In a further embodiment, exogenous HRG will not significantly reverse this inhibition.

To identify growth inhibitory ADC compounds that specifically target HER2, one may screen for ADC which inhibit the growth of HER2-overexpressing cancer cells derived from transgenic animals (US 5677171). According to this assay, HER2 overexpressing cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35mm dish) and the test compound is added at various concentrations. After six days, the number of cells, compared to untreated cells is counted using an electronic

COULTER™ cell counter. Those ADC which inhibit cell growth by about 20-100% or about 50-100% may be selected as growth inhibitory compounds.

To select for ADC which induce cell death, loss of membrane integrity as indicated by, e.g., PI, trypan blue or 7AAD uptake may be assessed relative to control. The PI uptake assay uses cells isolated from the breast tumor tissue of a transgenic animal. According to this assay, the 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. The cells are seeded at a density of 3 x 106 per dish in 100 x 20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing various concentrations of the compound. The cells are incubated for a 3-day time period. Following each 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 may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing compounds.

In order to select for compounds which induce apoptosis, an annexin binding assay using cells established from the breast tumor tissue of the transgenic animal is performed. The cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 µg/ml of the antibody-drug conjugate (ADC). 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 above for the cell death assay. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 µg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing compounds.

IN VITRO CELL PROLIFERATION ASSAYS

Generally, the cytotoxic or cytostatic activity of an antibody-drug conjugate (ADC) is measured by: exposing mammalian cells having tumor-associated antigens or receptor proteins to the antibody of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based *in vitro* assays were used to measure viability, i.e. proliferation (IC_{50}), cytotoxicity (EC_{50}), and induction of apoptosis (caspase activation) of the ADC.

The *in vitro* potency of antibody-drug conjugates was measured by a cell proliferation assay (Figures 1-4). The CellTiter-Glo® Luminescent Cell Viability Assay is a commercially available (Promega Corp., Madison, WI), homogeneous assay method based on the recombinant expression of *Coleoptera* luciferase (US 5583024; US 5674713; US 5700670). This cell proliferation assay determines the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells (Crouch et al (1993) J. Immunol. Meth. 160:81-88; US 6602677). The CellTiter-Glo® Assay was conducted in 96 well format,

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making it amenable to automated high-throughput screening (HTS) (Cree et al (1995) AntiCancer Drugs 6:398-404). The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent and mixing.

The homogeneous "add-mix-measure" format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiter-Glo® Assay generates a "glow-type" luminescent signal, produced by the luciferase reaction, which has a half-life generally greater than five hours, depending on cell type and medium used. Viable cells are reflected in relative luminescence units (RLU). The substrate, Beetle Luciferin, is oxidatively decarboxylated by recombinant firefly luciferase with concomitant conversion of ATP to AMP and generation of photons. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch mode processing of multiple plates. This cell proliferation assay an be used with various multiwell formats, e.g. 96 or 384 well format. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is presented as relative light units (RLU), measured over time.

$$\text{ATP} + \text{Luciferin} + O_2 \xrightarrow[Mg^{+2}]{\text{Luciferase}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + CO_2 + \text{light}$$

The anti-proliferative effects of three antibody-drug conjugates were measured by the cell proliferation, *in vitro* cell killing assay above against four different breast tumor cell lines (Figures 1-4). Figure 1 shows the potency measurements at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on SK-BR-3 (HER2 3+) breast tumor cells. Figure 2 shows the potency measurements of at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on BT-474 (HER2 3+) breast tumor cells. Figure 3 shows the potency measurements of at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on MCF7 (HER2 low) breast tumor cells. Figure 4 shows the potency measurements at increasing concentrations of trastuzumab-SPP-DM1, trastuzumab-SPDP-DM1 and trastuzumab-SMCC-DM1 after a 3 day treatment on MDA-MB-468 (HER2 negative) breast tumor cells.

IC_{50} values were established for SK-BR-3 and BT-474 which are known to overexpress HER2 receptor protein. A lot of conjugate trastuzumab-SPP-DM1 with 2.8 DM1 per trastuzumab (drug/Ab) gave a mean IC_{50} of 14.4 μg/ml with a range 9.1 to 22.3 μg/ml for 6 experiments against SK-BR-3 cells and a mean IC_{50} of 51.7 μg/ml with a range 28.7 to 63.1 μg/ml for 4 experiments against BT-474 cells. A lot of conjugate trastuzumab-SMCC-DM1 with 2.7 DM1 per trastuzumab (drug/Ab) gave a mean IC_{50} of 15.2 μg/ml with a range 12.6 to 18.8 μg/ml for 4 experiments against SK-BR-3 cells and a mean IC_{50} of 94.9 μg/ml with a range 75.2 to 114.6 μg/ml for 2 experiments against BT-474 cells. The conjugates were inactive against cells MCF7 and MDA-MB-468 which do not overexpress HER2.

AntiCD19-SMCC-DM1 showed potent cell killing *in vitro* with Raji cells (IC_{50} = <0.25 μg/ml) where the naked antibody and control ADC, trastuzumab-SMCC-DM1 showed no effect. AntiCD79a-SMCC-DM1 and antiCD79b-SMCC-DM1 showed potent cell killing in vitro with Ramos cells (IC_{50} = <0.25 μg/ml) where the naked antibody and control ADC, trastuzumab-SMCC-DM1 showed no effect.

Figure 17 shows an *in vitro*, cell proliferation assay (Example 5) with HT1080EphB2 (C8) cells treated with antiEphB2R 2H9 antibody-drug conjugates: 2H9-SPP-DM1 (IC_{50} 80 ng/ml), and 2H9-SMCC-DM1 (IC_{50} 50 ng/ml).

IN VIVO SERUM CLEARANCE AND STABILITY IN MICE

Serum clearance and stability of ADC were investigated in nude, naive (without tumors received by exogenous grafts) mice. Figure 5 shows the serum clearance in beige nude mice without tumors of trastuzumab-SMCC-DM1 vs. trastuzumab-SPP-DM1, measuring conjugate and total antibody serum concentration at six time points over 7 days. A difference in the amount of total antibody and ADC indicates cleavage of the linker and separation of the antibody from its DM1 moiety. As illustrated by the higher conjugated antibody serum concentration at seven days, SMCC-linked ADC remained intact *in vivo* longer than did SPP-linked conjugates.

Figure 6 shows the stability over time in nude mice without tumors of the conjugates: trastuzumab-SPDP-DM1, trastuzumab-SPP-DM1, trastuzumab-SPP-DM3, trastuzumab-SPP-DM4, and trastuzumab-SMCC-DM1, measuring serum concentration at six time points over 7 days. ADC with the SMCC linker were more stable *in vivo* than were conjugates linked by SPP or SPDP, although trastuzumab-SMCC-DM1 had approximately the same stability as the most hindered disulfide conjugate, trastuzumab-SPP-DM4.

The experiments shown in Figures 5 and 6 were performed in nude mice without tumors. However, SMCC-linked conjugates showed the same increased stability compared to SPP-linked conjugates in nude mice with tumors. As shown in Figure 7, approximately 72% of the initial trastuzumab-SMCC-DM1 remained as a conjugate 7 days after treatment, while only approximately 10% of the initial trastuzumab-SPP-DM1 remained as a conjugate 7 days after treatment, illustrating the improved stability of non-enzymatically cleavable trastuzumab-SMCC-DM1 conjugates in nude mice with tumors.

IN VIVO SERUM CLEARANCE AND STABILITY IN RATS

Figures 8 and 9 show the comparative stability and clearance profiles of a disulfide linker ADC (trastuzumab-SPP-DM1 and a non-disulfide linker ADC (trastuzumab-SMCC-DM1) in rats. The parameters of the study include:

Parameter	trastuzumab-SPP-DM1	trastuzumab-SMCC-DM1
Vd (ml/kg	41	41
Clearance (ml/day/kg)	52	15
T ½ alpha	0.09	0.15
T ½ beta	0.7	0.85
T ½ gamma (days)	2.6	5.5

The non-disulfide linker ADC, trastuzumab-SMCC-DM1 (Figure 9), showed better stability in rat serum than the disulfide linker ADC, trastuzumab-SPP-DM1 (Figure 8).

IN VIVO EFFICACY

The efficacy of the antibody-drug conjugates of the invention may be measured *in vivo* by implanting allografts or xenografts of cancer cells in rodents and treating the tumors with ADC. Variable results are to be expected depending on the cell line, the specificity of antibody binding of the ADC to receptors present on the cancer cells, dosing regimen, and other factors. The *in vivo* efficacy of anti-HER2 ADC was measured by a high expressing HER2 transgenic explant mouse model. An allograft was propagated from the Fo5 mmtv transgenic mouse which does not respond to, or responds poorly to, HERCEPTIN therapy. Subjects were treated once with ADC and monitored over 3-6 weeks to measure the time to tumor doubling, log cell kill, and tumor shrinkage. Follow up dose-response and multi-dose experiments were conducted.

Tumors arise readily in transgenic mice that express a mutationally activated form of *neu*, the rat homolog of HER2, but the HER2 that is overexpressed in breast cancers is not mutated and tumor formation is much less robust in transgenic mice that overexpress nonmutated HER2 (Webster et al (1994) Semin. Cancer Biol. 5:69-76).

To improve tumor formation with nonmutated HER2, transgenic mice were produced using a HER2 cDNA plasmid in which an upstream ATG was deleted in order to prevent initiation of translation at such upstream ATG codons, which would otherwise reduce the frequency of translation initiation from the downstream authentic initiation codon of HER2 (for example, see Child et al (1999) J. Biol. Chem. 274: 24335-24341). Additionally, a chimeric intron was added to the 5' end, which should also enhance the level of expression as reported earlier (Neuberger and Williams (1988) Nucleic Acids Res. 16: 6713; Buchman and Berg (1988) Mol. Cell. Biol. 8: 4395; Brinster et al (1988) Proc. Natl. Acad. Sci. USA 85:836). The chimeric intron was derived from a Promega vector, pCI-neo mammalian expression vector (bp 890-1022). The cDNA 3'-end is flanked by human growth hormone exons 4 and 5, and polyadenylation sequences. Moreover, FVB mice were used because this strain is more susceptible to tumor development. The promoter from MMTV-LTR was used to ensure tissue-specific HER2 expression in the mammary gland. Animals were fed the AIN 76A diet in order to increase susceptibility to tumor formation (Rao et al (1997) Breast Cancer Res. and Treatment 45:149-158).

Figures 10-13 show that the ADC have strong anti-tumor activity in the allograft of a HER2 positive tumor (Fo5) that originally arose in an MMTV-HER2 transgenic mouse. The antibody alone (e.g. trastuzumab) does not have significant anti-tumor activity in this model (Erickson et al US 6632979). As illustrated in

Figures 10 and 11, the growth of the tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth. Tumor growth was slowed most by treatment with trastuzumab-SMCC-DM1 and trastuzumab-SIAB-DM1 conjugates. As shown in Figures 10, 12 and 13, the trastuzumab-SMCC-DM1 conjugate slowed tumor growth more than conjugates with SPP linkers, i.e. more potency, whether measured as doubling time of tumors in nude mice or as Log Cell Kill corresponding to the doubling time measurements shown (Figure 13).

The *in vivo* efficacy of anti-CD22 ADC was measured with a mouse tumor xenograft model. Groups of eight SCID mice with 20 million Bjab-luc (luciferase expressing Bjab cells) xenograft tumor cells per mouse were dosed once at day 1 (except where noted) with an anti-CD22 antibody-drug conjugate, or a naked antibody (Example 8).

ADC or Ab	μg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
Tr-SMCC-DM1	200	4.2	3.2	3
7A2-SMCC-DM1	200	3.8	3.6	6
5E8-SMCC-DM1	200	3.8	3.6	10
RFB4-SMCC-DM1	200	3.2	4.25	18
RFB4-SMCC-DM1 (dosed 3X at 1, 7, 14 days)	405	10	2.75	55
7A2	-	4	-	3
5E8	-	4	-	3
RFB4	-	4	-	3

The time for tumor size to double was measured (MTD, mean tumor doubling time). The three naked anti-CD22 antibodies showed essentially no efficacy relative to a non-specific binding ADC (trastuzumab-SMCC-DM1). The corresponding conjugates all showed the effect of significantly retarding tumor growth. The effect of multiple dosing was established with RFB4-SMCC-DM1 where the MTD for singly dosed mice was 18 days whereas the MTD for mice dosed three times, at days 1, 7, and 14, was 55 days. Complete remission of tumor occurred in all 8 mice in the triple-dosed group. Other antiCD22-SMCC-DM1 conjugates, with antibodies 12F7, 9A8, 8C9, 8G10, 3F11, 10D2, 6C9, 14D1, and 11H10, showed shrinkage of initial tumor volume or retardation of tumor growth relative to control (trastuzumab-SMCC-DM1), after 7 days following a single dose (400 μg DM1/m2) in SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse. AntiCD22 conjugates, RFB4-SMCC-DM1, 5E8-SMCC-DM1, and 7A2-SMCC-DM1 were also effective in retardation of tumor growth relative to control (trastuzumab-SMCC-DM1), after 11 days following a single dose (200 μg DM1/m2) in SCID mice with 5 million Ramos RA1 xenograft tumor cells per mouse.

The conjugate RFB4-SMCC-DM1 was studied at three different drug loadings on groups of ten SCID mice with Bjab-luc xenografts (Example 8). The low (1.95) and medium (3.7) drug loaded conjugates each

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showed the effect of significantly retarding tumor growth, with an MTD of about 15 days. The high loaded (6.75) conjugate did not show an effect significantly different than control conjugate GP120-SMCC-DM1, or naked antibody RFB4.

anti-CD22 ADC or Ab	μg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
RFB4	-	10	-	3
RFB4-SMCC-DM1 (low loaded)	144	5	1.95	15
RFB4-SMCC-DM1 (medium loaded)	273	5	3.7	15
RFB4-SMCC-DM1 (high loaded)	497	5	6.75	3
GP120-SMCC-DM1 (high loaded)	449	5	6.1	3

AntiCD19-SMCC-DM1 and antiCD22-SMCC-DM1 conjugates did not show *in vivo* activity in a Raji cell mouse tumor xenograft model. Other antiCD19 and antiCD22 conjugates may have *in vivo* activity against other cancer cell tumor models.

The *in vivo* efficacy of anti-CD79a (alpha) and anti-CD79b (beta) ADC was measured with a mouse tumor xenograft model. Groups of eight SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with samples in the table below and following Example 8.

ADC, Ab, or control	μg DM1/m2	Ab mg / kg mouse	average drug loading (p)	MTD (days)
PBS (buffer control)	-	-	-	3.5
antiGP120	-	3.2	-	3.5
SN8 antiCD79b	-	3.1	-	4
17A7 antiCD79b	-	3.1	-	4
8H9 antiCD79a	-	4.0	-	3
antiGP120-SMCC-DM1	200	3.2	4.2	3.5
SN8 antiCD79b-SMCC-DM1	200	3.1	4.4	>7
17A7 antiCD79b-SMCC-DM1	200	3.1	4.4	>7
8H9 antiCD79a-SMCC-DM1	200	4.0	3.4	>7

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Conjugates SN8 antiCD79b-SMCC-DM1, 17A7 antiCD79b-SMCC-DM1, and 8H9 antiCD79a-SMCC-DM1, all showed shrinkage of initial tumor volume (mean 160 mm3) after 7 days. In the groups of 8 mice, conjugate SN8 antiCD79b-SMCC-DM1 gave partial remission (PR) in 4 animals and complete remission (CR) in 2 animals. Conjugate 17A7 antiCD79b-SMCC-DM1 gave CR in 1 animal. Conjugate 8H9 antiCD79a-SMCC-DM1 gave PR in 2 animals and CR in 1 animal. Other antiCD79b-SMCC-DM1 conjugates, with antibodies 2F2, 5C3, 7H7, 8D11, 15E4, and 16C11, showed retardation of tumor growth or shrinkage of initial tumor volume, relative to control (trastuzumab-SMCC-DM1), after 8 days following a single dose (192 μg DM1/m2) in CB17 ICR SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse.

The dose response effect on mice administered with antiCD79b-SMCC-DM1 was measured. Groups of eight SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with samples in the table below (Example 8). The antiCD79b-SMCC-DM1 was dosed at levels of 0.5, 2.0, and 3.64 mg Ab/kg mouse.

ADC, Ab, or control	μg DM1/m2	Ab mg / kg mouse	MTD (days)
Vehicle control (PBS)	-		4
antiCD79b-SMCC-DM1	32	0.5	10
antiCD79b-SMCC-DM1	130	2.0	35
antiCD79b-SMCC-DM1	236	3.64	>70

The *in vivo* efficacy of antiTENB2 ADC was measured with a mouse tumor xenograft model. TENB2 is a tumor antigen that is shown to be almost exclusively expressed in the human prostate and overexpressed in human prostate tumors (Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84). PC3-TVA-919cv1:5 is a human prostate cancer cell line expressing high level of TENB2.

Athymic nude mice were subcutaneously injected with 5 million PC3-TVA-919 high expressor or medium expressor cells in a volume of 0.2 ml per mouse. Cells were suspended in HBSS. When mean tumor size reached 100-200 mm^3, the mice were randomly grouped into 8 groups of 8-10 mice each and give a single IV treatment of the samples below (Example 8).

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ADC or control	µg DM1/m2	Ab mg / kg mouse	medium expressor MTD (days)	high expressor MTD (days)
PBS (vehicle control)	-	-	30	22
murine anti-ragweed-SPP-DM1 (negative control)	373	6.68	18	22
murine anti-ragweed-SMCC-DM1 (negative control)	373	9.34	14	18
murine antiTENB2:3146-SPP-DM1	373	7.34	58	35
murine antiTENB2:3146-SMCC-DM1	373	10.6	44	19
chimeric antiTENB2-SPP-DM1	373	8.7	57	43
chimeric antiTENB2-SMCC-DM1	373	7.63	43	22

Murine anti-TENB2-DM1 conjugates showed anti-tumor efficacy against PC3-TENB2 tumors, relative to negative control and vehicle control. Murine 10H1 antiNaPi3b-SMCC-DM1 conjugate showed no anti-tumor efficacy against PC3-NaPi3b tumors, relative to negative control and vehicle control. Other antibody variants of antiNaPi3b conjugates may have in vivo activity against PC3-NaPi3b tumors, or other cancer cell lines.

RODENT TOXICITY

Antibody-drug conjugates and an ADC-minus control, "Vehicle", were evaluated in an acute toxicity rat model. Toxicity of ADC was investigated by treatment of female Sprague-Dawley rats with the ADC and subsequent inspection and analysis of the effects on various organs. Based on gross observations (body weights), clinical pathology parameters (serum chemistry and hematology) and histopathology, the toxicity of ADC may be observed, characterized, and measured. It was found that at equivalent dose levels, trastuzumab-SMCC-DM1 was associated with less acute toxicity than trastuzumab-SPP-DM1.

A 5-day acute toxicity study in adolescent female rats (100-125 gms) was conducted by a single injection of trastuzumab-SMCC-DM1 (two doses: 1860 and 3260 µg DM1/m^2), a comparison disulfide ADC, trastuzumab-SPP-DM1 (two doses: 1860 and 3260 µg DM1/m^2), free DM1 maytansine (thiol) and a control Vehicle (day 0). Body weight was measured daily. Clinical chemistry, serum enzymes and hematology analysis was conducted on days 3 and 5; concluding with complete necropsy with histopathological assessment. Toxicity signals included the clinical observation of weight loss.

It is considered that weight loss, or weight change relative to animals dosed only with Vehicle in animals after dosing with ADC, is a gross and general indicator of systemic or localized toxicity. Figure 14 shows the changes in body weight (grams) over 5 days. Rats receiving the disulfide ADC, trastuzumab-SPP-DM1 showed a marked, dose-dependent toxicity, indicated by lethality at the higher dose and a decline in body

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weight at the lower dose. In contrast, rats receiving trastuzumab-SMCC-DM1 gained weight, with the lower dosed rats showing no decline in the rate of weight gain, relative to the placebo Vehicle-dosed rats. Rats dosed at the higher level of trastuzumab-SMCC-DM1 also gained weight, comparable to the free DM1 cytotoxin.

Hepatotoxicity was measured by elevated liver enzymes, increased numbers of mitotic and apoptotic figures and hepatocyte necrosis. Hematolymphoid toxicity was observed by depletion of leukocytes, primarily granuloctyes (neutrophils), and/or platelets, and lymphoid organ involvement, i.e. atrophy or apoptotic activity. Toxicity was also noted by gastrointestinal tract lesions such as increased numbers of mitotic and apoptotic figures and degenerative entercolitis.

Enzymes indicative of liver injury that were studied include:

AST (aspartate aminotransferase)

–Localization: cytoplasmic; liver, heart, skeletal muscle, kidney

–Liver:Plasma ratio of 7000:1

–T1/2: 17 hrs

ALT (alanine aminotransferase)

–Localization: cytoplasmic; liver, kidney, heart, skeletal muscle

–Liver:Plasma ratio of 3000:1

–T1/2: 42 hrs; diurnal variation

GGT (g-glutamyl transferase)

–Localization: plasma membrane of cells with high secretory or absorptive capacity; liver, kidney, intestine

–Poor predictor of liver injury; commonly elevated in bile duct disorders

None of the three enzymes measured above are liver-specific. It was found that the ADC of the present invention caused a transient, mild elevation of liver enzymes ALT and AST, with transient reticulocytopenia (Figure 15). No significant effect on peripheral blood granulocytes or platelets was observed (Figure 16).

Figures 15 and 16 show that rats exposed to 22.3 mg/kg trastuzumab-SPP-DM1, (Group 2) demonstrated the most severe clinical toxicity in this five day acute toxicity study. These animals showed the most profound loss of body weight, elevations of liver function tests, leuko- and thrombocytopenia and morphologic evidence of toxicity against hematolymphoid tissues. The extent of toxicity was similar compared to that encountered in previous studies using a dose of 25mg/kg. In contrast, animals in Groups 3 and 4 given trastuzumab-SMCC-DM1 at 10 and 25 mg/kg, respectively, were indistinguishable from vehicle-treated animals based on clinical pathology and body weight data. Morphologically, these animals showed a mildly increased number of mitotic figures in the liver, however, peripheral lymphoid and hematopoietic tissues were within normal limits.

Group 5 animals, 50 mg/kg trastuzumab-SMCC-DM1, showed evidence of toxicity. However, with the exception of one liver function test (ALT), the severity of toxicity was less than in animals receiving 50% of the same drug dose as trastuzumab-SPP-DM1 (Group 2). At about the same dose, trastuzumab-SMCC-DM1 (Group 4, 22.3 mg/kg) showed about 25% of the AST level as trastuzumab-SPP-DM1 (Group 2, 25 mg/kg). By day 5 of this study, Group 5 animals showed increases in body weights (following a transitory loss during days 3 and 4), a decreasing serum bilirubin and a rising platelet count (Figure 15).

Animals exposed to free maytansinoid DM1 (Group 6) show the same pattern of toxicity as animals treated with trastuzumab conjugates. This dose of free DM1 corresponds to the amount of drug given as a 10 mg/kg trastuzumab-SPP-DM1 dose in previous studies. The severity of toxicity in animals of group 6 was less than that seen in animals of Group 2, but more than previously seen in animals treated with 10 mg/kg trastuzumab-SPP-DM1. Recovery appeared to be rather fast: The sections of spleen showed increased numbers of immature hematopoietic elements in animals treated with free maytansine; also, LFTs and clinical hematology parameters showed a distinct trend towards normalization in animals of Group 6 by day 5.

CYNOMOLGUS MONKEY TOXICITY/SAFETY

Toxicity and safety of ADC administered to Cynomolgus monkeys may be assessed. A toxicity/safety study of the antibody-drug conjugate, trastuzumab-SMCC-DM1, was conducted in Cynomolgus monkeys. Three groups of monkeys were studied to assess the toxicity of trastuzumab-SMCC-DM1 administered via intravenous injection at escalating doses, relative to control (Vehicle). Group 1 (4 subjects) received only Vehicle (PBS, pH 6.5, i.e. formulation minus ADC) at day 1 and day 22, followed by necropsy at day 36. Group 2 (4 subjects) received trastuzumab-SMCC-DM1 4900 μg/m2 at day 1 and at day 22. Group 3 (4 subjects) received trastuzumab-SMCC-DM1 7200 μg/m2 at day 22.

Hepatotoxicity was inferred by measurement of the elevated liver enzymes from the Rodent Toxicity study. Cynomolgus monkeys were dosed with Vehicle (Group 1) and trastuzumab-SMCC-DM1 (Group 2: 4900 $\mu g/m^2$; Group 3: 7200 $\mu g/m^2$. Liver enzyme AST, platelet counts, white blood cells, absolute neutrophil, red blood cells, reticulocytes, and a comparison of 2 IV dose regimens were measured for trastuzumab-SMCC-DM1 in Cynomolgus Monkeys (Example 10).

ADMINISTRATION OF ANTIBODY-DRUG CONJUGATE PHARMACEUTICAL FORMULATIONS

Therapeutic antibody-drug conjugates (ADC) 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, bolus, intratumor injection or epidural (Shire et al (2004) J. Pharm. Sciences 93(6):1390-1402). Pharmaceutical formulations of therapeutic antibody-drug conjugates (ADC) are typically prepared for parenteral administration with a pharmaceutically acceptable parenteral vehicle and in a unit dosage injectable form. An antibody-drug conjugate (ADC) having the desired degree of purity is optionally mixed with pharmaceutically acceptable diluents, carriers, excipients or stabilizers, in the form of a lyophilized formulation or an aqueous solution (Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.).

Acceptable parenteral vehicles, diluents, carriers, excipients, and stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose,

mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). For example, lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein by reference. An exemplary formulation of an ADC such as trastuzumab-SMCC-DM1 contains about 100 mg/ml of trehalose (2-(hydroxymethyl)-6-[3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-tetrahydropyran-3,4,5-triol; $C_{12}H_{22}O_{11}$; CAS Number 99-20-7) and about 0.1% TWEEN™ 20 (polysorbate 20; dodecanoic acid 2-[2-[3,4-bis(2-hydroxyethoxy)tetrahydrofuran-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl ester; $C_{26}H_{50}O_{10}$; CAS Number 9005-64-5) at approximately pH 6.

Pharmaceutical formulations of a therapeutic antibody-drug conjugate (ADC) may contain certain amounts of unreacted drug moiety (D), antibody-linker intermediate (Ab-L), and/or drug-linker intermediate (D-L), as a consequence of incomplete purification and separation of excess reagents, impurities, and by-products, in the process of making the ADC; or time/temperature hydrolysis or degradation upon storage of the bulk ADC or formulated ADC composition. For example, a formulation of the ADC, trastuzumab-SMCC-DM1 may contain a detectable amount of free drug DM1. Alternatively, or in addition to, it may contain a detectable amount of drug-linker intermediate DM1-SMCC. Alternatively, or in addition to, it may contain a detectable amount of the antibody, trastuzumab. An exemplary formulation of trastuzumab-SMCC-DM1 may contain up to 10% molar equivalent of DM1-SMCC. Unexpectedly, it was determined by the *in vitro* cellular proliferation assay (Example 5), that DM1-SMCC (IC_{50} 0.05 μM) is about 20 times less potent in cell killing than free drug DM1 (IC_{50} 0.0045 μM) against SK-BR-3 and BT-474 breast cancer cells.

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

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the ADC, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (US 3773919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

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

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in *Remington's Pharmaceutical Sciences* (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared

by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Aqueous suspensions contain the active materials (ADC) in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, croscarmellose, povidone, methylcellulose, hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

The pharmaceutical compositions of ADC may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur. Subcutaneous (bolus) administration may be effected with about 1.5 ml or less of total volume and a concentration of about 100 mg ADC per ml. For ADC that require frequent and chronic administration, the subcutaneous route may be employed, such as by pre-filled syringe or autoinjector device technology.

As a general proposition, the initial pharmaceutically effective amount of ADC administered per dose will be in the range of about 0.01-100 mg/kg, namely about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range of compound used being 0.3 to 15 mg/kg/day. For example, human patients may be initially dosed at about 1.5 mg ADC per kg patient body weight. The dose may be escalated to the maximally tolerated dose (MTD). The dosing schedule may be about every 3 weeks, but according to diagnosed condition or response, the schedule may be more or less frequent. The dose may be further adjusted during the course of treatment to be at or below MTD which can be safely administered for multiple cycles, such as about 4 or more.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

Although oral administration of protein therapeutics are generally disfavored due to poor bioavailability due to limited absorption, hydrolysis or denaturation in the gut, formulations of ADC suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the ADC.

The formulations may be packaged in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water, for injection immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Exemplary unit dosage formulations contain a daily dose or unit daily sub-dose, or an appropriate fraction thereof, of the active ingredient.

The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefore. Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered parenterally, orally or by any other desired route.

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, such as cancer and autoimmune conditions. Exemplary conditions or disorders include benign or malignant tumors; leukemia and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic disorders. Cancer susceptible to ADC treatment include those which are characterized by the overexpression of certain tumor associated antigens or cell surface receptors, e.g. HER2

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 similar to the clinical trials testing the efficacy of the anti-HER2 monoclonal antibody HERCEPTIN in patients with HER2 overexpressing metastatic breast cancers that had received extensive prior anti-cancer therapy as reported by Baselga et al. (1996) J. Clin. Oncol. 14:737-744. The clinical trial may be designed to evaluate the efficacy of an ADC in combination with known therapeutic regimens, such as radiation and/or chemotherapy involving known chemotherapeutic and/or cytotoxic agents (Pegram et al (1999) Oncogene 18:2241-2251).

Generally, the disease or disorder to be treated is cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, gastrointestinal stromal tumor (GIST), pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

The cancer to be treated herein may be one characterized by excessive activation of an ErbB receptor, e.g. HER2. Such excessive activation may be attributable to overexpression or increased production of the ErbB receptor or an ErbB ligand. In one embodiment, a diagnostic or prognostic assay will be performed to determine whether the patient's cancer is characterized by excessive activation of an ErbB receptor. For example, ErbB gene amplification and/or overexpression of an ErbB receptor in the cancer may be determined. Various assays for determining such amplification/overexpression are available in the art and include the IHC, FISH and shed antigen assays described above. Alternatively, or additionally, levels of an ErbB ligand, such as TGF-alpha., in or associated with the tumor may be determined according to known procedures. Such assays may detect protein and/or nucleic acid encoding it in the sample to be tested. In one embodiment, ErbB ligand levels in the tumor may be determined using immunohistochemistry (IHC); see, for example, Scher et al. (1995) Clin. Cancer Research 1:545-550. Alternatively, or additionally, one may evaluate levels of ErbB ligand-encoding nucleic acid in the sample to be tested; e.g. via FISH, southern blotting, or PCR techniques. In one embodiment, ErbB2 overexpression may be analyzed by IHC, e.g. using the HERCEPTEST (Dako). Parrafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a ErbB2 protein staining intensity criteria as follows: Score 0, no staining is observed or membrane staining is observed in less than 10% of tumor cells; Score 1+, a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells, the cells are only stained in part of their membrane; Score 2+, a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells; Score 3+, a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells. Those tumors with 0 or 1+ scores for ErbB2 overexpression assessment may be characterized as not overexpressing ErbB2, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing ErbB2.

Alternatively, or additionally, FISH assays such as the INFORM™ (Ventana Co., Ariz.) or PATHVISION™ (Vysis, Ill.) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of ErbB2 overexpression in the tumor.

Moreover, ErbB receptor or ErbB ligand overexpression or amplification may be evaluated using an *in vivo* diagnostic assay, e.g. by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (e.g. a radioactive isotope) and externally scanning the patient for localization of the label.

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 the 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) 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, aromatase inhibitor, protein kinase inhibitor, lipid kinase inhibitor, anti-androgen, antisense oligonucleotide, ribozyme, gene therapy vaccine, anti-angiogenic 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 may also have a therapeutically effective amount of a chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, or a DNA binder.

Alternatively, or additionally, the second compound may be an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor. The second antibody may be monoclonal antibody 2C4 or humanized 2C4 "Omnitarg" (WO 01/00245). The second antibody may be conjugated with a cytotoxic or chemotherapeutic agent, e.g., a maytansinoid, an auristatin, a calicheamicin, or a 1,8 bis-naphthalimide moiety. For example, it may be desirable to further provide antibodies which bind to EGFR, ErbB2, ErbB3, ErbB4, or vascular endothelial factor (VEGF) in the one formulation or dosing regimen.

Other therapeutic regimens may be combined with the administration of an anticancer agent identified in accordance with this invention. 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 there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one embodiment, treatment with an ADC of the present invention 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, optionally along with treatment with an anti-ErbB2 antibody, such as trastuzumab. Chemotherapeutic agents include Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI), taxanes (such as paclitaxel and doxetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers's 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 anticancer agent may be combined with an anti-hormonal compound; e.g., an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (EP 616812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is hormone independent cancer, the patient may previously have been subjected to anti-hormonal therapy and, after the cancer becomes hormone independent, the anti-ErbB2 antibody (and optionally other agents as described herein) may be

administered to the patient. It may be beneficial to also coadminister a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

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 may be 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.

Metabolites include the products of *in vivo* cleavage of the ADC where cleavage of any bond occurs that links the drug moiety to the antibody. Metabolic cleavage may thus result in the naked antibody, or an antibody fragment. The antibody metabolite may be linked to a part, or all, of the linker. Metabolic cleavage may also result in the production a drug moiety or part thereof. The drug moiety metabolite may be linked to a part, or all, of the linker.

ARTICLES OF MANUFACTURE

In another embodiment, an article of manufacture, or "kit", containing ADC and 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, or blister pack. 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. In one embodiment, the label or package inserts indicates that the composition comprising the antibody which binds ErbB2 can be used to treat cancer which expresses an ErbB receptor selected from the group consisting of epidermal growth factor receptor (EGFR), ErbB2, ErbB3 and ErbB4. In addition, the label or package insert may indicate that the patient to be treated is one having cancer characterized by excessive activation of an ErbB receptor selected from EGFR, ErbB2, ErbB3 or ErbB4. For example, the cancer may be one which overexpresses one of these receptors and/or which overexpresses an ErbB ligand (such as TGF-α). The label or package insert may also indicate that the composition can be used to treat cancer, wherein the cancer is not characterized by overexpression of the ErbB2 receptor. In other embodiments, the package insert may indicate that the ADC composition can be used also to treat hormone independent cancer, prostate cancer, colon cancer or colorectal cancer.

The article of manufacture may comprise (a) a first container with a compound contained therein, wherein the compound comprises an ADC of the present invention in which the antibody of the ADC is a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second container with a compound contained therein, wherein the compound comprises a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, or a conjugate of this second antibody with a maytansinoid. The article of manufacture in this embodiment may further comprise a package insert indicating that the first and second compounds can be used to treat 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.

EXAMPLES

Example 1 - Production, Characterization and Humanization of Anti-ErbB2 Monoclonal Antibody 4D5

The murine monoclonal antibody 4D5 which specifically binds the extracellular domain of ErbB2 was produced as described in Fendly et al (1990) Cancer Research 50:1550-1558. Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al (1987) Proc. Natl. Acad. Sci. (USA) 84:7158-7163 were harvested with phosphate buffered saline (PBS) containing 25mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P-labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13.

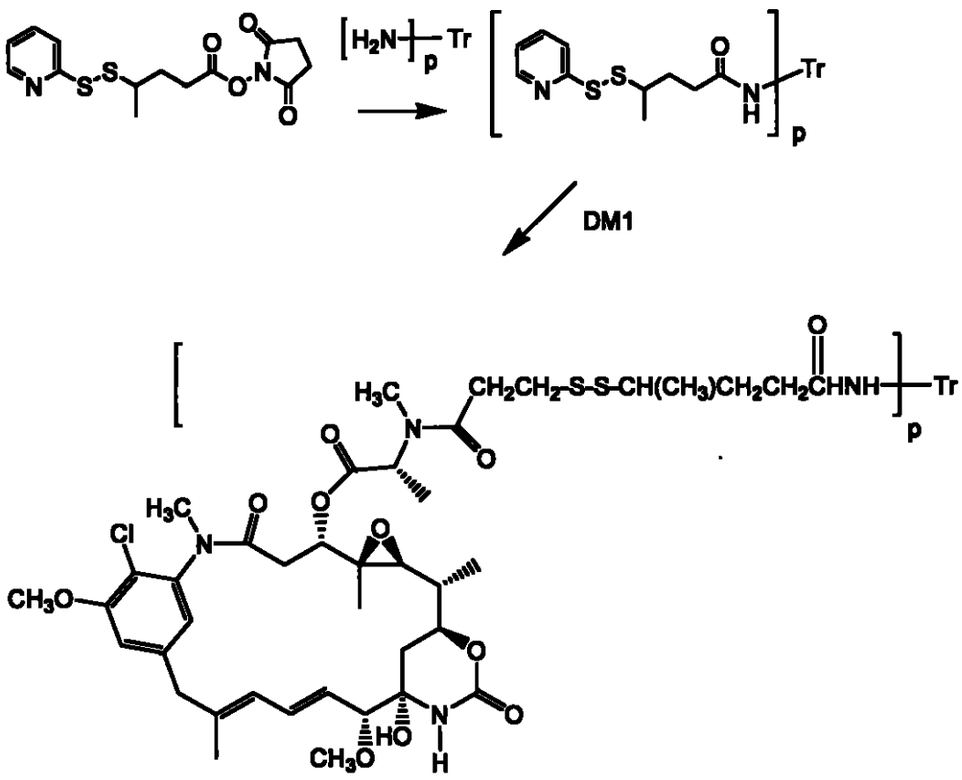
This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

The murine monoclonal antibody 4D5 was humanized, using a "gene conversion mutagenesis" strategy, as described in US 5821337, the entire disclosure of which is hereby expressly incorporated by reference. The humanized monoclonal antibody 4D5 used in the following experiments is designated huMAb4D5-8. This antibody is of IgG1 isotype.

Example 2 - Purification of Trastuzumab

One vial containing 440 mg HERCEPTIN® (huMAb4D5-8, rhuMAb HER2, US 5821337) antibody) was dissolved in 50 mL MES buffer (25 mM MES, 50 mM NaCl, pH 5.6) and loaded on a cation exchange column (Sepharose S, 15 cm x 1.7 cm) that had been equilibrated in the same buffer. The column was then washed with the same buffer (5 column volumes). Trastuzumab was eluted by raising the NaCl concentration of the buffer to 200 mM. Fractions containing the antibody were pooled, diluted to 10 mg/mL, and dialyzed into a buffer containing 50 mm potassium phosphate, 50 mM NaCl, 2 mM EDTA, pH 6.5.

Example 3 - Preparation of Antibody-Drug Conjugates: Trastuzumab-SPP-DM1:



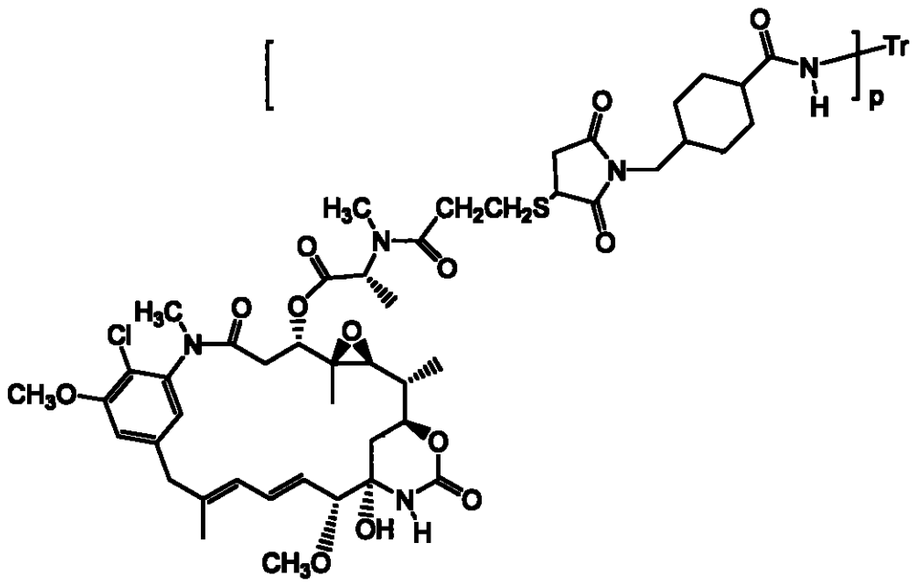
Purified trastuzumab was derivatized with N-succinimidyl-4-(2-pyridylthio)pentanoate to introduce dithiopyridyl groups. Trastuzumab (376.0 mg, 8 mg/mL) in 44.7 mL of 50 mM potassium phosphate buffer (pH 6.5) containing NaCl (50 mM) and EDTA (1 mM) was treated with SPP (5.3 molar equivalents in 2.3 mL

ethanol). After incubation for 90 minutes under argon at ambient temperature, the reaction mixture was gel filtered through a Sephadex G25 column equilibrated with 35 mM sodium citrate, 154 mM NaCl, 2 mM EDTA. Antibody containing fractions were pooled and assayed. The degree of modification of the antibody was determined as described above. Recovery of the modified antibody (trastuzumab-SPP-Py) was 337 mg (89.7%) with 4.5 releasable 2-thiopyridine groups linked per antibody (p').

Trastuzumab-SPP-Py (337.0 mg, 9.5 μmols of releasable 2-thiopyridine groups) was diluted with the above 35 mM sodium citrate buffer, pH 6.5, to a final concentration of 2.5 mg/mL. DM1 ($N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl)-maytansine), the structure of which is shown in Figure 1, (1.7 equivalents, 16.1 μmols) in 3.0 mM dimethylacetamide (DMA, 3% v/v in the final reaction mixture) was then added to the antibody solution. The reaction proceeded at ambient temperature under argon for 20 hours.

The reaction was loaded on a Sephacryl S300 gel filtration column (5.0 cm x 90.0 cm, 1.77 L) equilibrated with 35 mM sodium citrate, 154 mM NaCl, pH 6.5. The flow rate was 5.0 mL/min and 65 fractions (20.0 mL each) were collected. A major peak centered around fraction No. 47 (Figure 3). The major peak comprises monomeric trastuzumab-SPP-DM1. Fractions 44-51 were pooled and assayed. The number of DM1 drug molecules linked per antibody molecule (p') was determined by measuring the absorbance at 252 nm and 280 nm, and found to be 3.7 drug molecules per antibody molecule.

Example 4 - Preparation of Antibody-Drug Conjugates: Trastuzumab-SMCC-DM1



Purified trastuzumab was derivatized with (Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate, SMCC, Pierce Biotechnology, Inc) to introduce the SMCC linker. Trastuzumab was purified from HERCEPTIN® as in Example 2 and buffer-exchange treated at 20 mg/mL in 50 mM potassium phosphate/ 50 mM sodium chloride/ 2 mM EDTA, pH 6.5 with 7.5 to 10 molar equivalents of SMCC (20 mM in DMSO or DMA (dimethylacetamide), 6.7 mg/mL). After stirring for 2 to 4 hours under argon at ambient temperature, the reaction mixture was filtered through a Sephadex G25 column equilibrated with 50mM potassium phosphate/ 50 mM sodium chloride/ 2 mM EDTA, pH 6.5. Alternatively, the reaction mixture was gel filtered with 30 mM

citrate and 150 mM sodium chloride at pH 6. Antibody containing fractions were pooled and assayed. Recovery of trastuzumab-SMCC was 88%.

The drug-linker intermediate, trastuzumab-SMCC from above, was diluted with 50 mM potassium phosphate/50 mM sodium chloride/2 mM EDTA, pH 6.5, to a final concentration of 10 mg/ml, and reacted with a 10 mM solution of DM1 (1.7 equivalents assuming 5 SMCC/trastuzumab, 7.37 mg/ml) in dimethylacetamide. The reaction was stirred at ambient temperature under argon for 4 to about 16 hours. The conjugation reaction mixture was filtered through a Sephadex G25 gel filtration column (1.5 x 4.9 cm) with 1 x PBS at pH 6.5. Alternatively, the reaction mixture was gel filtered with 10 mM succinate and 150 mM sodium chloride at pH 5. The DM1/trastuzumab ratio (p) was 3.1, as measured by the absorbance at 252 nm and at 280 nm. The drug to antibody ratio (p) may also be measured by mass spectrometry. Conjugation may also be monitored by SDS polyacrylamide gel electrophoresis. Aggregation may be assessed by laser light scattering analysis.

Antibody-SMCC-DM1 Conjugates:

Following this protocol, other antibody-drug conjugates with the SMCC linker and DM1 drug moiety were prepared, including:

Antibody-drug conjugate (ADC)	antibody (Ab)	antigen	average drug loading (DM1/Ab)
10H-SMCC-DM1	10H1 (murine) anti-NaPi3b	NaPi3b	5.0
2H9-SMCC-DM1	2H9 Mab	anti-EphB2R	4.0
ragweed-SMCC-DM1	ragweed		2.7
antiTENB2-SMCC-DM1	anti-TENB2-3416#2 Mouse MAb	TENB2	2.5
anti-TENB2 20D1 Fc Chimera-SMCC-DM1	Chimeric Human Mouse anti-TENB2	TENB2	3.32
antiCD19-SMCC-DM1	antiCD19 (mIgG1 isotype)	CD19	5.05
2H7-SMCC-DM1	antiCD20 (mIgG2a isotype)	CD20	2.44, 3.84 (two lots)
CD20 LC-SMCC-DM1	antiCD20 (Rituxan®)	CD20	
HB5-SMCC-DM1	antiCD21 (mIgG2a isotype)	CD21	4.05
RFB4-SMCC-DM1	antiCD22	CD22	1.95-6.75 (multiple lots)
Cy34.1.2-SMCC-DM1	antiCD22	CD22	2.70
12F7.1.5-SMCC-DM1	antiCD22	CD22	5.90
9A8.1.1-SMCC-DM1	antiCD22	CD22	5.00

8C9.1.2-SMCC-DM1	antiCD22	CD22	2.90
14B3.3.1-SMCC-DM1	antiCD22	CD22	5.20
8G10.4.2-SMCC-DM1	antiCD22	CD22	3.60
2B4.1.4-SMCC-DM1	antiCD22	CD22	5.20
7A2.4.1-SMCC-DM1	antiCD22	CD22	3.60
4H3.2.2-SMCC-DM1	antiCD22	CD22	3.60
5E8.1.8-SMCC-DM1	antiCD22	CD22	3.60
3F11.2.1-SMCC-DM1	antiCD22	CD22	4.50
6C9.1.3-SMCC-DM1	antiCD22	CD22	3.70
10D2.4.3-SMCC-DM1	antiCD22	CD22	3.65
10D6.8.1-SMCC-DM1	antiCD72	CD72	2.90
ZL7-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
5C3-SMCC-DM1	antiCD79a (alpha)	CD79a	1.85
6G1-SMCC-DM1	antiCD79a (alpha)	CD79a	3.50
7H7-SMCC-DM1	antiCD79a (alpha)	CD79a	3.25
8D11-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
8H9-SMCC-DM1	antiCD79a (alpha)	CD79a	3.40
11E5-SMCC-DM1	antiCD79a (alpha)	CD79a	3.60
15E4-SMCC-DM1	antiCD79a (alpha)	CD79a	3.25
16C11-SMCC-DM1	antiCD79a (alpha)	CD79a	2.50
2F2-SMCC-DM1	antiCD79b (beta)	CD79b	3.20
17A7-SMCC-DM1	antiCD79b (beta)	CD79b	4.40
SN8-SMCC-DM1	antiCD79b (beta)	CD79b	4.40, 0.80 (two lots)
1F9-SMCC-DM1	antiFcRH1	FcRH1	4.20
7A2-SMCC-DM1	antiFcRH2	FcRH2	4.20, 3.95 (two lots)
7G7-SMCC-DM1	antiFcRH2	FcRH2	3.80
cl1D6-SMCC-DM1	antiFcRH2	FcRH2	2.20
51505.111-SMCC-DM1	antiCXCR5	CXCR5	6.10

12C7 Fc Chimera-SMCC-DM1	antiBrevican	brevican	4.60
H2:PSCA hlog-SMCC-DM1	antiPSCA hlog	PSCA	3
H6:xPSCAhlog-SMCC-DM1	antiPSCA hlog	PSCA	2.6
xFcRH5:3909 7D11.1.1-SMCC-DM1	antiIRTA2	IRTA2	2.6
xFCRH5.7D11-SMCC-DM1	antiIRTA2	IRTA2	4.4
11D10 LC-SMCC-DM1	Mouse anti-CA 0772P MAb	CA 0772P MUC16	
xNCA LC-SMCC-DM1	Mouse anti-CEACAM6 MAb	CEACAM6 NCA	

Antibody-BMPEO-DM1 Conjugates:

For cysteine conjugation, the antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, in the presence of EDTA. After incubating for about one hour at 37 °C, the pH is adjusted to about 7 with 100 mM potassium phosphate. The reduced antibody is modified by the bis-maleimido reagent BM(PEO)4 (Pierce Chemical), leaving an unreacted maleimido group on the surface of the antibody. This may be accomplished by dissolving BM(PEO)4 in a 50% ethanol/water mixture to a concentration of 10 mM and adding a tenfold molar excess to a solution containing antibody in phosphate buffered saline at a concentration of approximately 1.6 mg/ml (10 μM) and allowing it to react for 1 hour to form Ab-BMPEO. Excess BM(PEO)4 is 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 is dissolved in dimethyl acetamide (DMA) and added to the Ab-BMPEO intermediate. Dimethyl formamide (DMF) may also be employed to dissolve the drug moiety reagent. The reaction mixture is allowed to react overnight before gel filtration or dialysis into PBS to remove unreacted DM1. Gel filtration on S200 columns in PBS was used to remove high molecular weight aggregates and furnish purified Ab-BMPEO-DM1.

Following this protocol, other antibody-drug conjugates with the BMPEO linker and DM1 drug moiety were prepared, including:

Antibody-drug conjugate (ADC)	antibody (Ab)	antigen	average drug loading (DM1/Ab)
trastuzumab-BM(PEO)-DM1	trastuzumab (Herceptin®)	HER2	2.94
CD120-BMPEO4-DM1	Mouse anti-GP120 MAb	GP120	2
RFB4-BMPEO4-DM1	antiCD22	CD22	3.7 and 4.25 (two lots)

Example 5 - In vitro cell proliferation assay

Efficacy of ADC were measured by a cell proliferation assay employing the following protocol (Promega Corp. Technical Bulletin TB288; Mendoza et al (2002) Cancer Res. 62:5485-5488):

1. An aliquot of 100 µl of cell culture containing about 10^4 cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.
2. Control wells were prepared containing medium and without cells.
3. ADC was added to the experimental wells and incubated for 3-5 days.
4. The plates were equilibrated to room temperature for approximately 30 minutes.
5. A volume of CellTiter-Glo Reagent equal to the volume of cell culture medium present in each well was added.
6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.
7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.
8. Luminescence was recorded and reported in graphs as RLU = relative luminescence units.

Example 6 - Serum clearance and stability in mice

A beige mutation, nude, naive (without tumors) mice in six Groups of four animals were studied. At Day 0, each mouse received a single 2 mg/kg dose of ADC in 200 µl aqueous carrier, except the Vehicle Group which received only carrier. Blood was collected by cardiac puncture under anesthesia at each time point (5 minutes, 1 hour, 6 hours, 24 hours, 72 hours, and 168 hours) post-dose. The serum was isolated and antibody and ADC were measured.

Group 1: Vehicle (PBS, pH 6.5, no ADC)
Group 2: Trastuzumab-SMCC-DM1
Group 3: Trastuzumab-SPP-DM1
Group 4: Trastuzumab-SPDP-DM1
Group 5: Trastuzumab-SPP-DM3
Group 5: Trastuzumab-SPP-DM4

Example 7 - Serum stability in rats

Six dose groups with 6 Sprague-Dawley rats (100-125 gms each) per dose group were studied. At Day 0, Animals were administered a single IV dose of Vehicle, 10 mg/kg trastuzumab-SPP-DM1, or 10 mg/kg trastuzumab-SMCC-DM1, at a dose volume of 10 ml/kg via the lateral tail vein. Approximately 300 µl whole blood was collected at each timepoint: 0 (predose), 10, and 30 minutes; 1, 2, 4, 8, 24 and 36 hours; and 2, 3, 4, 7, 14, 21, 28 days post dose.

Example 8 - Tumor volume in vivo efficacy

High expressing HER2 transgenic explant mice:

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but FVB female mice are preferred because of their higher susceptibility to tumor formation. FVB males were used for mating and vasectomized CD.1 studs were used to stimulate pseudopregnancy. Vasectomized mice can be obtained from any commercial supplier.

Founders were bred with either FVB mice or with 129/BL6 x FVB p53 heterozygous mice. The mice with heterozygosity at p53 allele were used to potentially increase tumor formation. However, this has proven unnecessary. Therefore, some F1 tumors are of mixed strain. Founder tumors are FVB only. Six founders were obtained with some developing tumors without having litters.

Animals having tumors (allograft propagated from Fo5 mmtv transgenic mice) were treated with a single injection of a trastuzumab-DM1 maytansinoid conjugate (10 mg/kg dose) and tumor volume was assessed for over 20 days after injection.

Bjab-luc xenograft SCID mice:

Groups of eight to ten SCID mice with 20 million Bjab-luc xenograft tumor cells per mouse were dosed at day 1 with an antibody-drug conjugate, or a naked antibody. A group of eight mice were tested each with anti-CD22 ADC, naked anti-CD22 antibodies, and control. Control ADC (trastuzumab-SMCC-DM1; loading: DM1/trastuzumab =3.2) is not a specific binder and was administered at 200 μg DM1/m2, 4.2 trastuzumab/kg mouse, and resulted in a mean tumor doubling time of about 3 days. Anti-CD22 conjugates 7A2-SMCC-DM1 (loading: DM1/Ab = 3.6), 5E8-SMCC- DM1 (loading: DM1/Ab = 3.6), and RFB4-SMCC-DM1 (loading: DM1/Ab = 4.3). Naked antibodies, 7A2, 5E8, and RFB4 were dosed at 4 mg/kg mouse.

Example 9 - Rat toxicity

The acute toxicity profile of trastuzumab-SPP-DM1 (disulfide linker) compared with that of free DM1 and trastuzumab-SMCC-DM1 (non-disulfide linker) was evaluated). Animals were injected on day 1, complete chemistry and hematology profiles were obtained at baseline, day 3 and day 5 and a complete necropsy was performed on day 5. Routine histology was performed on three random animals for each group for the following tissues: sternum, liver, kidney, thymus, spleen, large and small intestine. The experimental groups were as follows:

Group 1: Vehicle (10 mM sodium succinate, 100 mg/mL sucrose, 0.1% Tween 20, pH 5.0)
Group 2: Trastuzumab-SPP-DM1, 22.3mg/kg
Group 3: Trastuzumab-SMCC-DM1, 10mg/kg
Group 4: Trastuzumab-SMCC-DM1, 25mg/kg
Group 5: Trastuzumab-SMCC-DM1, 50mg/kg
Group 6: Free DM1, 160μg/kg

Example 10 - Cynomolgus Monkey Toxicity/Safety

Three groups of four (2 male, 2 female) naive *Macaca fascicularis* (cynomolgus monkey) were studied.

Group 1: (4 animals) received only Vehicle (PBS, pH 6.5, i.e. formulation minus ADC) at day 1 and day 22, followed by necropsy at day 36.
Group 2: (4 animals) received trastuzumab-SMCC-DM1 4900 μg/m2 at day 1 and at day 22.
Group 3: (4 animals) received trastuzumab-SMCC-DM1 7200 μg/m2 at day 22.

Dosing is expressed in surface area of an animal so as to be relevant to other species, i.e. dosage at μg/m2 is independent of species and thus comparable between species. Formulations of trastuzumab-SMCC-

DM1 for Group 2 and Group 3 studies contained PBS, 5.4mM sodium phosphate, 4.2 mM potassium phosphate, 140 mM sodium chloride, pH 6.5.

Blood was collected for hematology analysis prior to first dosing (acclimation period) and at days 3, 7, 11, and 14 after the first dose (Groups 1 and 2) and at days 3, 7, 11, 14, and 21 after the second dose (Groups 1, 2 and 3). Erythrocyte (RBC) and platelet (PLT) counts were measured by the light scattering method. Leukocyte (WBC) count was measured by the peroxidase/basophil method. Reticulocyte count was measured by the light scattering method with cationic dye. Cell counts were measured on an Advia 120 apparatus. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in U/L by UV/NADH; IFCC methodology on an Olympus AU400 apparatus, and using Total Ab ELISA - ECD/GxhuFc-HRP. Conj. Ab ELISA - xDM1/ECD-Bio/SA-HRP tests.

All patents, patent applications, and references cited throughout the specification are expressly incorporated by reference.

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We claim:

1. An antibody-drug conjugate compound comprising an antibody covalently attached by a linker to one or more maytansinoid drug moieties, the compound having Formula I:

$$Ab–(L–D)_p \qquad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

provided that the antibody is not TA.1;

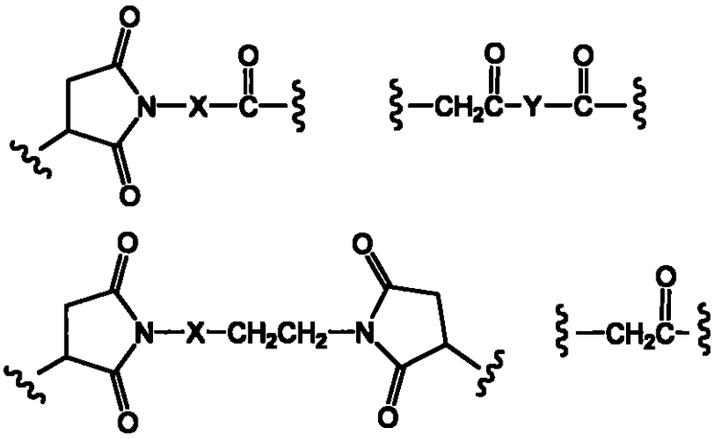
L is a non-disulfide linker;

D is a maytansinoid drug moiety; and

p is 1 to 8.

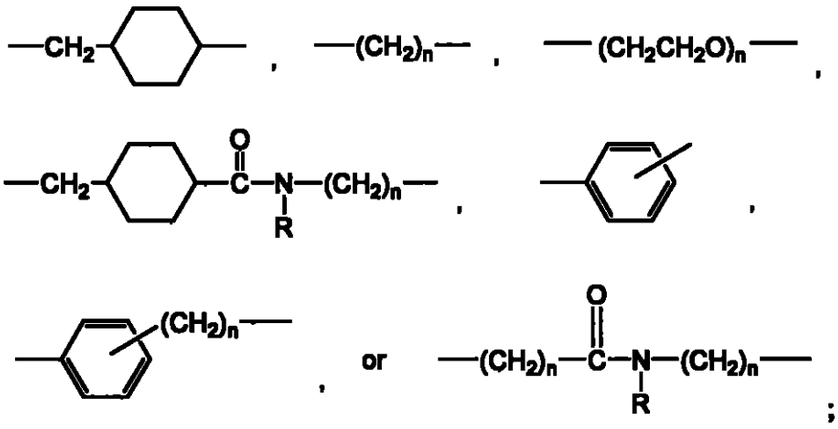
2. The antibody-drug conjugate compound of claim 1 wherein Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36).

3. The antibody-drug conjugate compound of claim 1 or claim 2 wherein L is a linker selected from the structures:

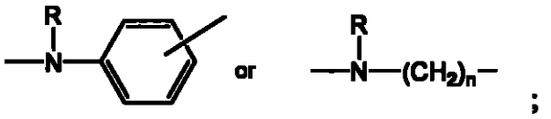


where the wavy lines indicate the covalent attachments to Ab and D;

X is:

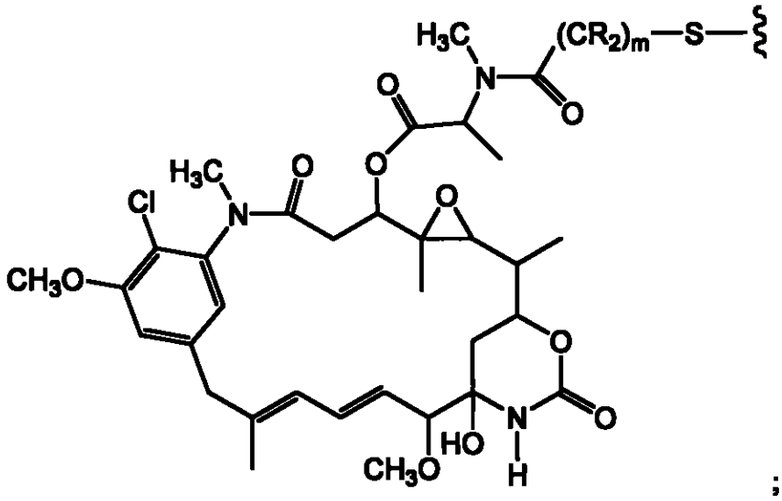


Y is:



R is independently H or C_1–C_6 alkyl; and n is 1 to 12.

4. The antibody-drug conjugate compound of any of the preceding claims wherein D is selected from the structure:



;

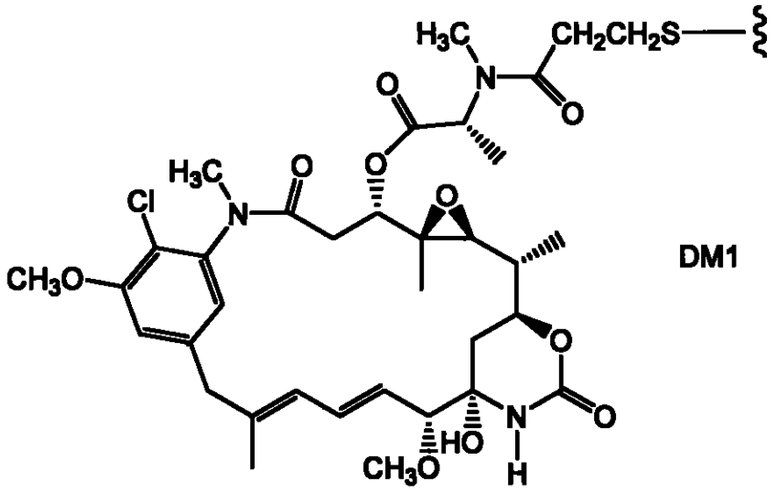
where the wavy line indicates the covalent attachment to L,

R is independently H or C_1–C_6 alkyl, and

m is 1, 2, or 3.

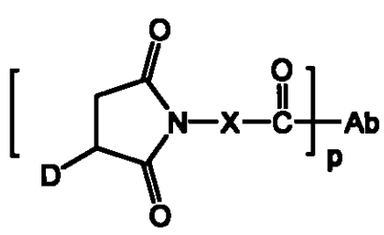
5. The antibody-drug conjugate compound of claim 4 wherein m is 2, and R is H.

6. The antibody-drug conjugate compound of any of the preceding claims wherein the maytansinoid drug moiety is DM1, having the structure:

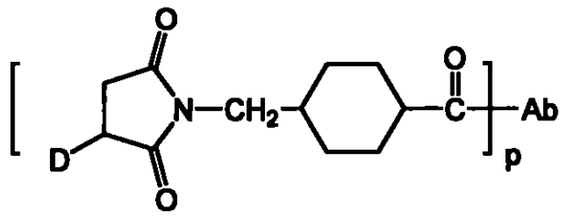


DM1

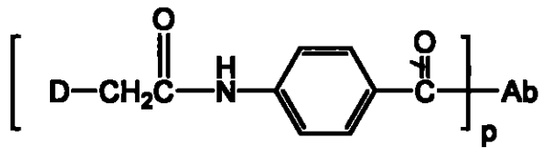
7. The antibody-drug conjugate compound of claim 3 having the structure:



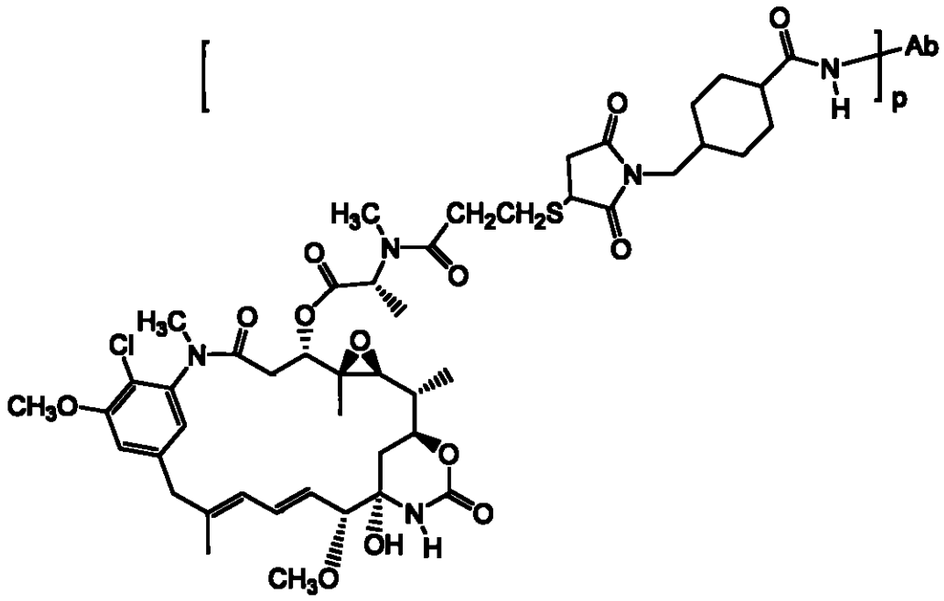
8. The antibody-drug conjugate compound of claim 7 having the structure:



9. The antibody-drug conjugate compound of claim 3 having the structure:

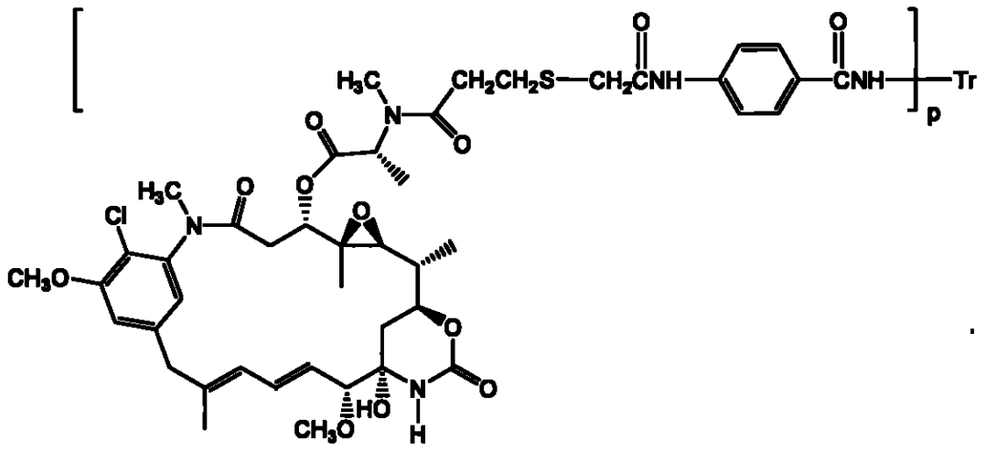


10. The antibody-drug conjugate compound of claim 1 having the structure:



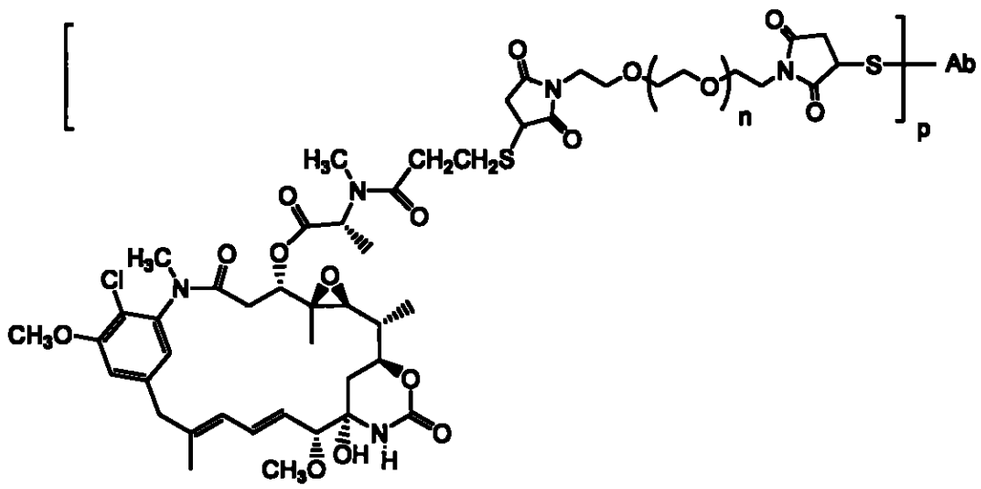
11. The antibody-drug conjugate of claim 10 wherein Ab is trastuzumab, and p is 1, 2, 3, or 4.

12. The antibody-drug conjugate compound of claim 1 having the structure:



where Tr is trastuzumab, and p is 1, 2, 3, or 4.

13. The antibody-drug conjugate compound of claim 1 having the structure:



wherein n is 0, 1, or 2; and p is 1, 2, 3, or 4.

14. The antibody-drug conjugate compound of claim 13 wherein Ab is trastuzumab.

15. The antibody-drug conjugate compound of claim 1 wherein p is 1, 2, 3, or 4.

16. The antibody-drug conjugate compound of claim 1 wherein the antibody binds to a receptor encoded by an ErbB gene.

17. The antibody-drug conjugate compound of claim 16 wherein the receptor is selected from EGFR, HER2, HER3 and HER4.

18. The antibody-drug conjugate compound of claim 17 wherein the antibody specifically binds to a HER2 receptor.

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19. The antibody-drug conjugate compound of claim 1 which specifically binds to the extracellular domain of the HER2 receptor and inhibits growth of tumor cells which overexpress HER2 receptor.

20. The antibody-drug conjugate compound of any of the preceding claims wherein the antibody is selected from a monoclonal antibody, an antibody fragment, a chimeric antibody, and a humanized antibody.

21. The antibody-drug conjugate compound of claim 1 wherein the antibody is a humanized antibody selected from huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (trastuzumab).

22. The antibody-drug conjugate compound of claim 21 wherein the antibody is huMAb4D5-8 (trastuzumab).

23. The antibody-drug conjugate of claim 20 wherein the antibody is a Fab fragment.

24. The antibody-drug conjugate compound of claim 1 wherein the antibody is attached to the linker through a cysteine thiol of the antibody.

25. The antibody-drug conjugate compound of claim 24 wherein p is 1, 2, 3, or 4.

26. A pharmaceutical composition comprising the antibody-drug conjugate compound of claim 1 or claim 2, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient.

27. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of a chemotherapeutic agent selected from Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozole, Imatinib mesylate, PTK787/ZK 222584, Oxaliplatin, 5-FU, Leucovorin, Rapamycin, Lapatinib, Lonafarnib, Sorafenib, and Gefitinib.

28. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of a anti-angiogenic agent.

29. The pharmaceutical composition of claim 26 further comprising a therapeutically effective amount of bevacizumab.

30. A method of inhibiting cellular proliferation comprising treating mammalian cells in a cell culture medium with an antibody-drug conjugate compound of claim 1 or claim 2, whereby proliferation of the cells is inhibited.

31. The method of claim 30 wherein the mammalian cells have HER2 receptor proteins to the antibody-drug conjugate compound.

32. The method of claim 31 wherein the mammalian cells are breast tumor cells.

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33. The method of claim 30 wherein the antibody-drug conjugate compound is more cytotoxic than a maytansinoid compound comprising the maytansinoid moiety of the antibody-drug conjugate compound.

34. The method of claim 30 wherein the antibody-drug conjugate induces apoptosis.

35. A method of treating cancer comprising administering to a patient a formulation of an antibody-drug conjugate compound of claim 1 or claim 2, and a pharmaceutically acceptable diluent, carrier or excipient.

36. The method of claim 35 wherein the cancer is selected from the group consisting of breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer.

37. The method of claim 36 wherein the cancer is breast cancer which overexpresses ErbB2 at a 2+ level or more.

38. The method of claim 35 wherein the amount of antibody-drug conjugate compound administered to the patient is in the range of about 0.1 to about 10 mg/kg of patient weight per dose.

39. The method of claim 35 wherein the antibody-drug conjugate is administered at about three week intervals.

40. The method of claim 35 wherein the antibody-drug conjugate is administered by infusion.

41. The method of claim 35 wherein the antibody-drug conjugate is formulated with a pharmaceutically acceptable parenteral vehicle.

42. The method of claim 41 wherein the antibody-drug conjugate is formulated in a unit dosage injectable form.

43. The method of claim 42 wherein the antibody-drug conjugate is administered intravenously.

44. The method of claim 35 wherein the patient is administered a growth inhibitory antibody, in combination with the antibody-drug conjugate compound.

45. The method of claim 35 wherein the patient is administered a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, in combination with the antibody-drug conjugate compound.

46. The method of claim 45 wherein the second antibody comprises monoclonal antibody 2C4 or humanized 2C4.

47. The method of claim 46 wherein said second antibody is conjugated with a cytotoxic agent.

48. The method of claim 35 wherein the patient is administered a chemotherapeutic agent, in combination with the antibody-drug conjugate compound, where the chemotherapeutic agent is selected from Erlotinib, Bortezomib, Fulvestrant, Sutent, Letrozole, Imatinib mesylate, PTK787/ZK 222584, Oxaliplatin, 5-FU, Leucovorin, Rapamycin, Lapatinib, Lonafarnib, Sorafenib, and Gefitinib.

49. The method of claim 35 wherein the patient is administered an anti-angiogenic agent in combination with the antibody-drug conjugate compound where the anti-angiogenic agent is selected from bevacizumab.

50. A method of inhibiting the growth of tumor cells that overexpress a growth factor receptor, comprising administering to a patient an antibody-drug conjugate of claim 1 which binds specifically to a growth factor receptor selected from HER2 or EGF and a chemotherapeutic agent wherein said antibody-drug conjugate and said chemotherapeutic agent are each administered in amounts effective to inhibit growth of tumor cells in the patient.

51. A method for the treatment of a human patient susceptible to or diagnosed with a disorder characterized by overexpression of ErbB2 receptor, comprising administering a combination of an antibody-drug conjugate of claim 1 and a chemotherapeutic agent or a growth inhibitory agent.

52. The method of claim 51 wherein the antibody of the antibody-drug conjugate is an anti-ErbB2 antibody.

53. The method of claim 52 wherein the anti-ErbB2 antibody has a biological characteristic of a 4D5 monoclonal antibody.

54. The method of claim 52 wherein the anti-ErbB2 antibody binds essentially the same epitope as a 4D5 monoclonal antibody.

55. The method of claim 52 wherein the anti-ErbB2 antibody is selected from the group consisting of humanized antibodies huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (trastuzumab).

56. An assay for detecting cancer cells comprising:

(a) exposing cells to an antibody-drug conjugate compound of claim 1 or claim 2; and

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

57. The assay of claim 56 wherein the cells are breast tumor cells.

58. The assay of claim 56 wherein the extent of binding is determined by measuring levels of ErbB-encoding nucleic acid by fluorescent in situ hybridization (FISH).

59. The assay of claim 56 wherein the extent of binding is determined by immunohistochemistry (IHC).

60. An article of manufacture comprising

an antibody-drug conjugate compound of claim 1 or claim 2;

a container; and

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

61. The article of manufacture of claim 60 wherein said package insert of label indicates that the compound can be used to treat cancer characterized by the overexpression of an ErbB2 receptor.

62. The article of manufacture of claim 61 wherein the cancer is breast cancer.

63. The article of manufacture of claim 62 wherein the cancer is characterized by the overexpression of an ErbB2 receptor at a 2+ level or above.

64. A method of making an antibody-drug conjugate compound comprising an antibody covalently attached by a linker to one or more maytansinoid drug moieties, the compound having Formula I:

$$Ab–(L–D)_p \qquad I$$

or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);

(17) HER2 (Genbank accession no. M11730);

(18) NCA (Genbank accession no. M18728);

(19) MDP (Genbank accession no. BC017023);

(20) IL20Rα (Genbank accession no. AF184971);

(21) Brevican (Genbank accession no. AF229053);

(22) EphB2R (Genbank accession no. NM_004442);

(23) ASLG659 (Genbank accession no. AX092328);

(24) PSCA (Genbank accession no. AJ297436);

(25) GEDA (Genbank accession no. AY260763;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, NP_443177.1);

(27) CD22 (B-cell receptor CD22-B isoform, NP-001762.1);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes, Genbank accession No. NP_002111.1);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis, Genbank accession No. NP_005573.1);

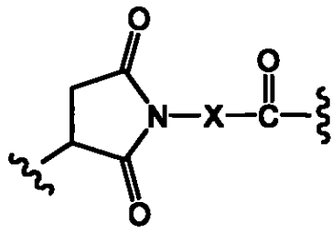
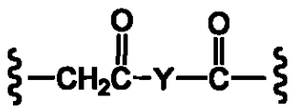
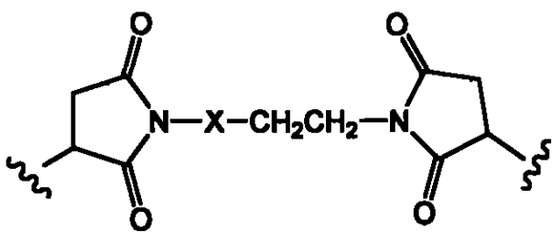
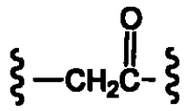
(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1); and

(36) TENB2 (putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin, Genbank accession No. AF179274;

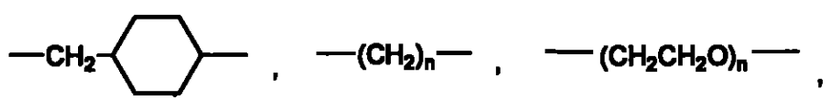
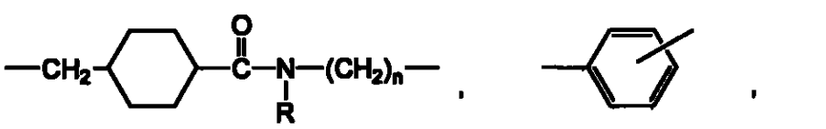
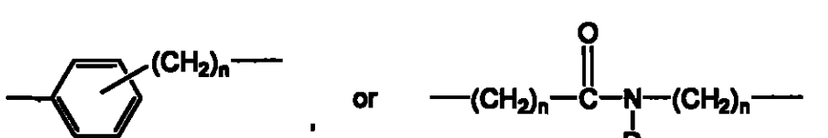
provided that the antibody is not TA.1;

L is a linker selected from the structures;

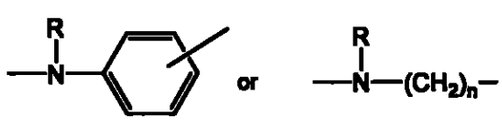





where the wavy lines indicates the covalent attachments to Ab and D;

X is:

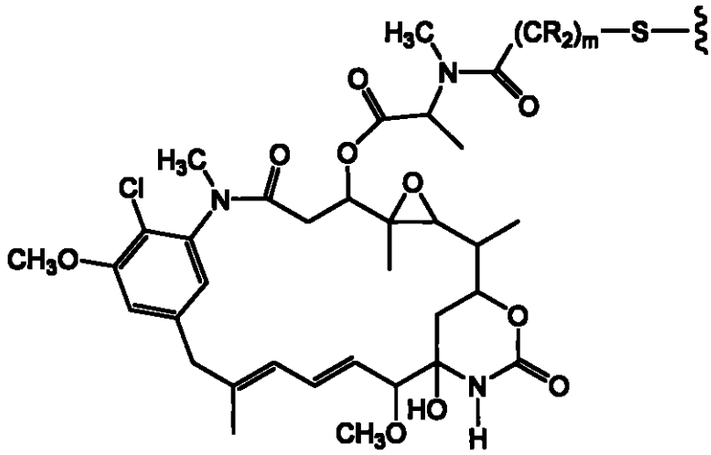




Y is:



where R is independently H or C_1–C_6 alkyl; and n is 1 to 12;

D is a maytansinoid drug moiety selected from the structure:



;

where the wavy line indicates the covalent attachment to L; R is independently H or C_1–C_6 alkyl; m is 1, 2, or 3; and p is 1 to 8;

wherein the method comprises:

reacting Ab with a linker reagent to form antibody-linker intermediate Ab-L, and then reacting Ab-L with a drug moiety D to form the antibody-drug conjugate; or

reacting a drug moiety D with a linker reagent to form a drug-linker intermediate D-L, and then reacting D-L with Ab to form the antibody-drug conjugate.

65. The method of claim 64 wherein Ab is an antibody which binds to one or more tumor-associated antigens or cell-surface receptors selected from (1)-(16) and (18)-(36).

66. The method of claim 64 or claim 65 wherein the linker reagent is SMCC.

67. The method of claim 64 or claim 65 wherein the linker reagent is a bis-maleimide reagent selected from DTME, BMB, BMDB, BMH, BMOE, $BM(PEO)_3$, and $BM(PEO)_4$.

ANTIBODY-DRUG CONJUGATES AND METHODS

ABSTRACT

The present invention relates to antibody-drug conjugate compounds of Formula I:

$$Ab–(L–D)_p \qquad I$$

where one or more maytansinoid drug moieties (D) are covalently linked by L to an antibody (Ab) which binds to an ErbB receptor, or which binds to one or more tumor-associated antigens or cell-surface receptors. These compounds may be used in methods of diagnosis or treatment of cancer, and other diseases and disorders.

UNITED STATES DEPARTMENT OF COMMERCE
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Washington, D.C. 20231

SEPTEMBER 24, 2004

PTAS

700117285A

GENENTECH, INC.
ALEX ANDRUS
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

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RECORDATION DATE: 09/23/2004 REEL/FRAME: 015168/0529
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
JACOBSON, FREDERIC S. DOC DATE: 09/09/2004

ASSIGNOR:
SCHWALL, RALPH H. DOC DATE: 09/01/2004

ASSIGNOR:
SLIWKOWSKI, MARK X. DOC DATE: 09/01/2004

ASSIGNOR:
SPENCER, SUSAN D. DOC DATE: 09/15/2004

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080-4990

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

RECORDATION FORM COVER SHEET
PATENTS ONLY

Atty Dkt No: PR2094

Assistant Commissioner of Patents: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

Frederic S. Jacobson
Ralph H. Schwall
Mark X. Sliwkowski
Susan D. Spencer

Additional name(s) of conveying party(ies) attached? _ Yes X No

2. Name and address of receiving party(ies):

Name: GENENTECH, INC.
Internal Address:
Street Address: 1 DNA Way
City: South San Francisco State: California
ZIP: 94080-4990

Additional name(s) & address(es) attached? _ Yes X No

3. Nature of conveyance:

X Assignment Merger
Security Agreement
Change of Name
Other:

Execution Date: 9/9/04; 9/1/04; 9/1/04; 9/15/04

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)
60/576,517

B. Patent No.(s)

Additional numbers attached? _ Yes X No

5. Name and address of party to whom correspondence concerning document should be mailed:

Alex Andrus
GENENTECH, INC.
1 DNA Way
South San Francisco, CA 94080

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41): $ 40.00
_ Enclosed
X Authorized to be charged to deposit account

8. Deposit account number:
07-0630

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Alex Andrus
Name of Person Signing
Registration No. 44,509

Signature

September 23, 2004
Date

Total number of pages comprising cover sheet, attachments and document: 3

Revised (10/1/95)

430

Docket No. PR2094

ASSIGNMENT

WHEREAS, Frederic S. Jacobson, a citizen of USA, residing at 830 Shattuck Ave., Berkeley, CA 94707, and Ralph H. Schwall, a citizen of USA, residing at 400 Griffen Ave., Pacifica, CA 94404, and Mark X. Silwkowski, a citizen of USA, residing at 42 Oak Creek Lane, San Carlos, CA 94070, and Susan D. Spencer, a citizen of USA, residing at 2395 Paradise Drive, Tiburon, CA 94920, (hereinafter "ASSIGNORS"), have invented a new and useful invention in

Antibody Drug Conjugates and Methods

for which a provisional application Serial No. 60/576,517 (Docket No. PR2094) has been filed by them on June 1, 2004; 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 94080-4490, 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 underaign as follows;

South San Francisco
Dated: 9/9/04

Frederic S. Jacobson

South San Francisco
Dated: Sept. 2004

Ralph H. Schwall

South San Francisco
Dated: Sept 1, 2004

Mark X. Sliwkowski

South San Francisco
Dated: 9/15/04

Susan D. Spencer

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
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Washington, D.C. 20231

700178803A

JULY 02, 2005

PTAS *700178803A*

ALEX ANDROS
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

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RECORDATION DATE: 05/11/2005 REEL/FRAME: 016214/0872
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
EBENS, ALLEN DOC DATE: 04/12/2005

ASSIGNOR:
JACOBSON, FREDERIC S. DOC DATE: 04/25/2005

ASSIGNOR:
POLAKIS, PAUL DOC DATE: 04/20/2005

ASSIGNOR:
SCHWALL, RALPH H. DOC DATE: 04/22/2005

ASSIGNOR:
SLIWKOWSKI, MARK X. DOC DATE: 04/21/2005

016214/0872 PAGE 2

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080-4990

SERIAL NUMBER: 60616098 FILING DATE: 10/05/2004
PATENT NUMBER: ISSUE DATE:
TITLE: ANTIBODY DRUG CONJUGATES AND METHODS

LAENA MARTIN, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

1

434

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

RECORDATION FORM COVER SHEET
PATENTS ONLY

Atty Dkt No: PR2094-1

Commissioner for Patents: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

Allen Ebens
Frederic S. Jacobson
Paul Polakis
Ralph H. Schwall
Mark X. Sliwkowski

Additional name(s) of conveying party(ies) attached? _ Yes X No

2. Name and address of receiving party(ies):

Name: GENENTECH, INC.

Internal Address:

Street Address: 1 DNA Way

City: South San Francisco State: California

ZIP: 94080-4990

Additional name(s) & address(es) attached? _ Yes X No

3. Nature of conveyance:

X Assignment Merger

Security Agreement

Change of Name

Other:

Execution Date: 04/12/05, 04/25/05, 04/20/05, 04/22/05, 04/22/05, 04/21/05

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)

60/616,098

B. Patent No.(s)

Additional numbers attached? _ Yes X No

5. Name and address of party to whom correspondence concerning document should be mailed:

Alex Andrus
GENENTECH, INC.
1 DNA Way
South San Francisco, CA 94080

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41): $ 40.00

_ Enclosed

X Authorized to be charged to deposit account

8. Deposit account number:
07-0630

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Alex Andrus
Name of Person Signing
Registration No. 44,509

Signature

May 11, 2005
Date

Total number of pages comprising cover sheet, attachments and document: 3

Revised (10/11/03)

434

435

Docket No. PR2094-1

ASSIGNMENT

WHEREAS, Allen Ebens, a citizen of USA, residing at 1932 Arroyo Ave., San Carlos, CA 94070, and Frederic S. Jacobson, a citizen of USA, residing at 830 Shattuck Ave., Berkeley, CA 94707 , and Paul Polakis, a citizen of USA, residing at 1449 Cortez Ave., Burlingame, CA 94010, and Ralph H. Schwall, a citizen of USA, residing at 400 Griffin Ave., Pacifica, CA 94044, and Mark X. Sliwkowski, a citizen of USA, residing at 42 Oak Creek Lane, San Carlos, CA 94070 and Susan D. Spencer, a citizen of USA, residing at 2395 Paradise Drive, Tiburon, CA 94920 (hereinafter "ASSIGNORS"), have invented a new and useful invention in

Antibody Drug Conjugates and Methods

for which a provisional application Serial No. 60/616,098 (Docket No. PR2094-1) has been filed by them on October 5, 2004; 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: 4/12/05

Allen Ebens

South San Francisco
Dated: 4/25/05

Frederic S. Jacobson

South San Francisco
Dated: 4/20/05

Paul Polakis

South San Francisco
Dated: 4-22-05

Ralph H. Schwall

South San Francisco
Dated: 4/22/05

Mark X. Sliwkowski

South San Francisco
Dated: 4/21/05

Susan D. Spencer

AB 99908

437

437

State of California

SECRETARY OF STATE

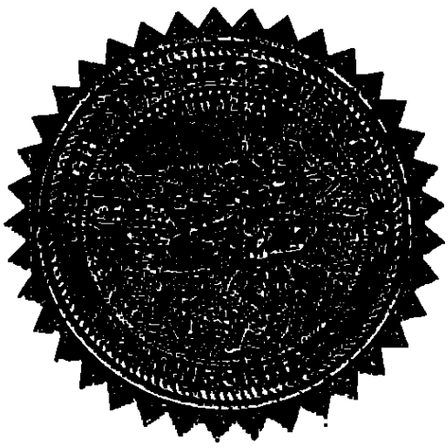
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(Convention de La Haye du 5 octobre 1961)

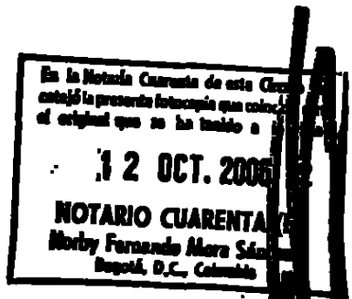
1. *Country:. United States of America*
   This public document
2. *has been signed by AIDA MICLAT GUIAM*
3. *acting in the capacity of Notary Public, State of California*
4. *bears the seal/stamp of AIDA MICLAT GUIAM, Notary Public, State of California*

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-94 A (REV. 4-06)

OSP 06 60772

4/37

4/38

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.

Aida Miclat Guiam
(Signature of Notary Public)

AIDA MICLAT GUIAM
Commission # 1456662
Notary Public - California
San Mateo County
My Comm. Expires Dec 9, 2007

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☐ Partner — ☐ Limited ☐ General
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12 OCT. 2006

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Norby Fernando Mora Sánchez
Bogotá, D.C., Colombia

Brigard & Castro
BOGOTA - COLOMBIA

439

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, declaraciones, 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 creyeran 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 200_

Firma

Nombre
Cargo

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 OCT. 2006

NOTARIO CUARENTA (E)
Norby Fernando Mora Sánchez
Bogotá, D.C., Colombia

P WER F 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 exploitation 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

6th 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.



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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.**

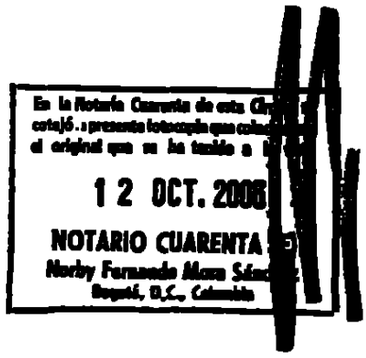
ESTADO DE CALIFORNIA
SECRETARIA DE ESTADO

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

CW2

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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 # 14556812
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

Descripción adicional de cualquier documento anexo

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Nombre del firmante: _____

☐ Individuo
☐ Funcionario Corporativo – Título(s): _____
☐ Socio - ☐ Limitado ☐ General
☐ Abogado de hecho
☐ Fideicomisario
☐ Guardián o conservador
☐ Otro: _____

El firmante representa: _____

De la Notaría Cuarenta de este círculo notarial se extendió la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 OCT. 2007
NOTARIO CUARENTA
Henry Fernando Mora Sánchez
Bogotá, D.C., Colombia

Huella índice derecho del firmante
Parte alta del dedo aquí

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AUTORIZACI N

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.



97. 200
1/4

044

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

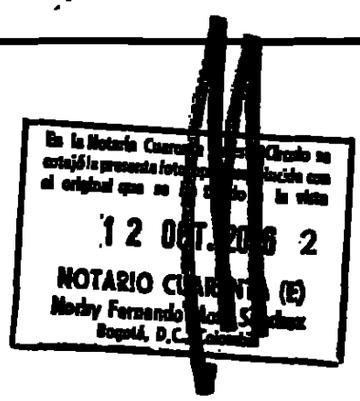
8. No. 0254030

9. Seal/Stamp:

10. Signature:

Harriet Smith Windsor

Secretary of State

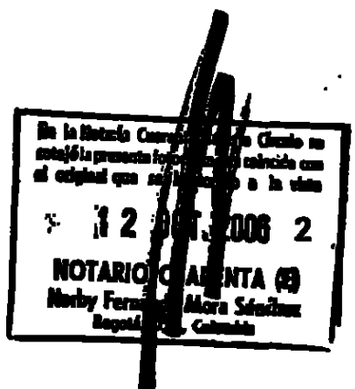


Delaware

945

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

050426506

AUTHENTICATION: 3900384

DATE: 05-24-05

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

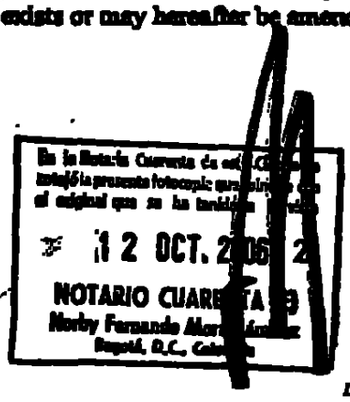
The name of the Corporation is: Genentech, Inc.

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

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

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Norby Fernando Castro Sánchez
Bogotá, D.C. Colombia

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.



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

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Norby Fernando Mora Sánchez
Bogotá, D.C., Colombia

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



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

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12 OCT. 2006
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Norby Fernando Mora [illegible]
Bogotá, D.C., Colombia

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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[illegible]
Bogotá, D.C.,

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 the

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Norby Fernando Mor[illegible]
Bogotá, D.C., Co[illegible]

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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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.

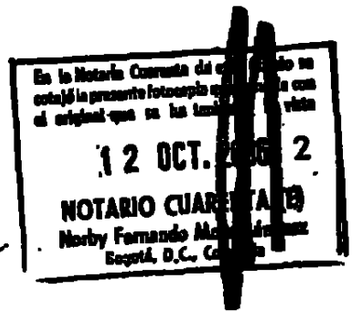


456

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



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TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc. 457

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

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12 OCT. 2005

NOTARIO CUARENTA (E)
Norby Fernando [illegible]
Bogotá, D.C. Colombia

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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 extrajo la presente fotocopia que coincide con el original que se le tuvo a la vista

12 OCT. 2005

NOTARIO CUARENTA (E)
Norby Fernando Mora Martínez
Bogotá, D.C., Colombia

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900384

FECHA: 05 – 24 – 05

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459

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**").

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 [illegible]nes

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NOTARIO CUARENTA (E)
Nadry Fernando Mora Sánchez
Bogotá, D.C., Colombia

CORREGIDO Y VUELTO A REDACTAR
CERTIFICADO DE INCORPORACIÓN
GENENTECH, INC.

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NOTARIO CUARENTA
Nerby Fernando Mora Sánchez
Bogotá, D.C., Colombia

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 de tal serien caso de una liquidación, disolución o cierre voluntaria o involuntaria de la Corporación y

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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.

o

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.

ARTICULO 6

SECCION 6.01. *Elección del Directorio por los Accionistas de [illegible]ación Preferencial.* Durante cualquier periodo cuando los accionistas de c[illegible]r

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NOTARIO CUARENTA (E)
Harby Fernando Mora Sánchez
Bogotá, D.C., Colombia

acción preferencial cualquiera de una o más series de las mismas, que voten como una clase, tengan derecho a elegir un número específico de directores, por razón a atrasos en los dividendos u otras condiciones que les dan el derecho a hacerlo, entonces y durante el tiempo que continúe el derecho (i) el número de directores autorizado de otro modo será incrementado por tal número de directores especificado, y los accionistas de tales Acciones Preferenciales o tales series de las mismas, que votan como una clase, tendrán derecho a elegir los directores adicionales para lo que se provee, de acuerdo con las condiciones de tales Acciones Preferenciales o series; (ii) cada uno de tales directores adicionales servirá durante tal termino, y tendrá tales poderes de voto, como se han de otorgar en las provisiones pertinentes a tales Acciones Preferenciales o series; y (iii) siempre que los accionistas de cualquiera de tales Acciones Preferenciales o series de las mismas están desprovistos de tales derechos a elegir un número específico de directores, votando como clase, de acuerdo con las condiciones de tales Acciones preferenciales o series, los términos de oficio de todos los directores elegidos por los accionistas de tales Acciones preferenciales o series, que votan como clase, de acuerdo con las condiciones o elegidos para llenar cualquier vacante resultante de la muerte, renuncia o remoción de directores así elegidos por los accionistas de tales Acciones Preferenciales o series, terminarán en adelante y el número autorizado de directores será reducido en concordancia.

SECCION 6.02. *Votaciones*. Las elecciones de directores en una reunión anual o especial de accionistas necesaria no serán por votos escritos a menos que los estatutos de la Corporación digan lo contrario.

SECCION 6.03. *Eliminación de cierta responsabilidad personal de los directores*. (a) Un director de la Corporación no será responsable ante la Corporación o sus accionistas por daños monetarios por violación del deber fiduciario como director hasta donde lo permita la Ley de Delaware.

(b) Cada persona (y sus herederos, ejecutores o administradores de tal persona) quien fue o es una parte o esta amenazado de ser parte de, o esta involucrado en cualquier acción, demanda o proceso amenazado, pendiente, o completado, ya sea civil, criminal, administrativo o investigativo, por razón del hecho de que esa persona es o fue un director de la Corporación o es o estaba sirviendo por solicitud de la Corporación como un director de otra corporación, sociedad, sociedad de riesgo compartido, fideicomiso u otra empresa, será indemnizado y completamente protegido por la Corporación hasta donde

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NOTARIO CUARENTA
Norby Fernando Mora Sánchez
Bogotá, D.C., Colombia

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.

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.

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Norby Fernando Mo...
Bogotá, D.C., Colombia

ARTICULO 8

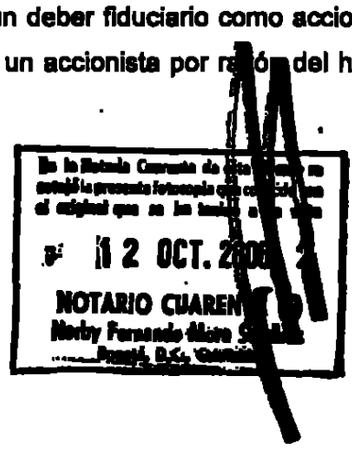
SECCION 8.01. *Relación con Roche.* Anticipándose a que la Corporación cese de ser un subsidiaria completamente de propiedad de Roche Holdings, Inc., pero que Roche (como se define en la sección 8.05) permanezca siendo un accionista de la Corporación, y anticipándose a que la Corporación (como se define en la Sección 8.05) y Roche puede entrar en actividades o lineas 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 lineas 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



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.

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

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Bogotá, D.C., Colombia

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 Certificado 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.

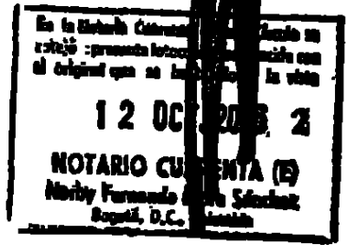
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NOTARIO CUARENTA
Norby Fernando Ávila Sánchez
Bogotá, D.C., Colombia

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.

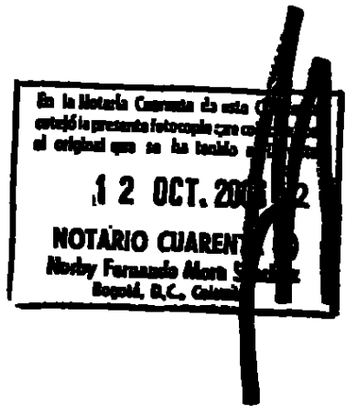


SECCION 9.06. *Efecto obligante.* Ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 9 ni la adopción de alguna condición inconsistente con cualquier condición de este Artículo 9 eliminará o reducirá el efecto de este Artículo 9 respecto a cualquier materia que 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

470

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 autenticó la presente fotocopia que coincide con el original que se ha tenido a la vista.

12 OCT. 2006

NOTARIO CUARENTA (E)

Norby Fernando Mora S.

Bogotá, D.C., Colombia

Delaware

471

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

050426506

AUTHENTICATION: 3900385

DATE: 05-24-05

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 05:00 PM 05/15/2000
001245851 – 2112549

472

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: _____
Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel and Secretary

75841

[illegible] 2006 2
NOTARIO [illegible]

TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc.

473

APOSTILLA

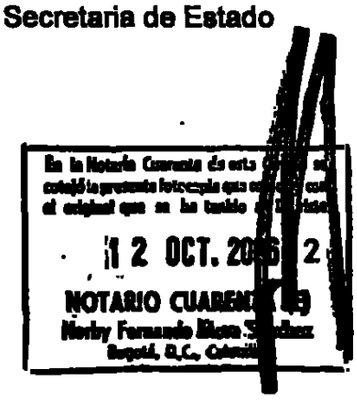
(Convención de La Haya del 5 de Octubre de 1961)

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



474

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 $0.02 por acción ("Acción Común"). La acción común de la Corporación será toda de una misma clase.

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.

En la Notaría [illegible] de esta Diligencia se cotejó la presente [illegible] que coincide con el original que [illegible] a la vista
[illegible] 2006 2
NOTARÍA CUARENTA (E)
Nasby Fernando Mora Sánchez
Bogotá D.C., Colombia

477

476

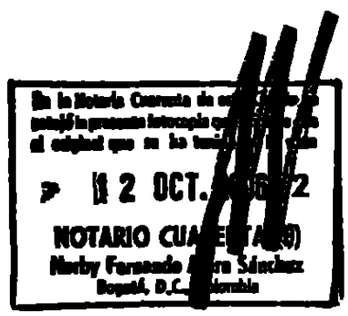
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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.



477

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

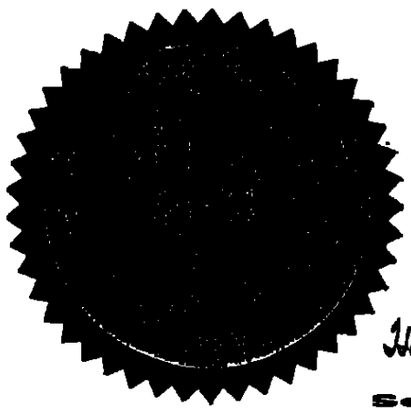
5. at Dover, Delaware

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 C... cotejó la presente fotocopia que coincide con el original que se le tuvo a la vista.

12 OCT. 200...

NOTARIO CUARENTA (E...

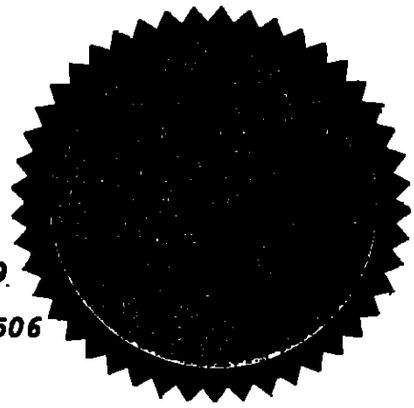
Norby Fernando Mora Sá...

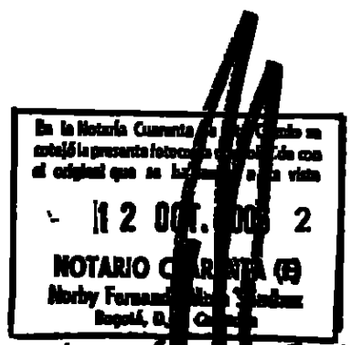
Bogotá, D.C., Colombia

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.





Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900387

DATE: 05-24-05

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

479

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 [illegible] se extrajó la presente fotocopia que [illegible] al original que se ha tenido [illegible]

12 OCT. 2[illegible]2

NOTARIO CUARE[illegible]

Norby Fernando Mor[illegible]

Bogotá, D.C., Col[illegible]

TRADUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

480

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

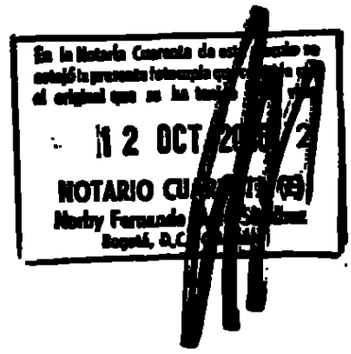


481

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.



Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900387

FECHA: 05 – 24 – 05

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.

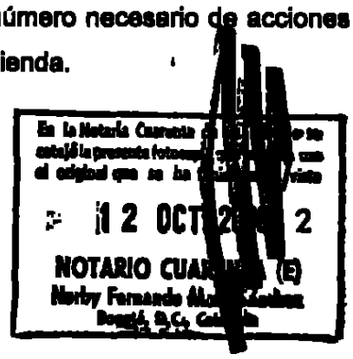
CERTIFICA POR LA PRESENTE:

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 millónes (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.

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.



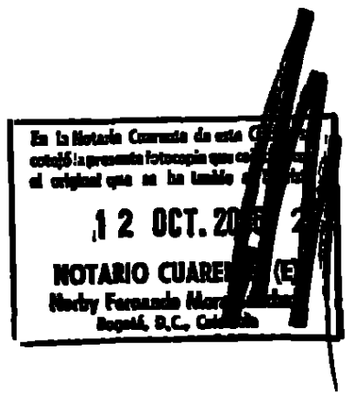
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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 16 de Abril de 2004.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.



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

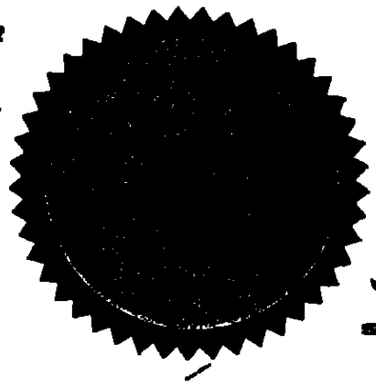
5. at Dover, Delaware

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 OCT. 2005

NOTARIO CUARENTA

Norby Fernando Mora Sil

Bogotá, D.C., Colombia

Delaware

485

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 [illegible] se extendió la presente fotocopia [illegible] el original que se [illegible]

12 OCT [illegible] 2

NOTARIO CUARENTA (E)

Norby Fernando [illegible]

Bogotá, D.C.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900386

DATE: 05-24-05

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 02:05 PM 05/10/2001
010220224 – 2112549

S ////STATE/OF/DELAWARE///// V
T ///SECRETARY/OF/STATE///// O
A DIVISION/OF/CORPORATIONS// I
M FILED/02:05/PM/05/10/2001/ D
P ///010220224/–/2112549//// BY Sandy Miller

486

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

867e7

En la Notaría Cuarenta (E) del Círculo se extiende la presente copia que coincide con el original que se tuvo a la vista
12 OCT 2006
NOTARIO CUARENTA (E)
Norby Fernando Mora Sánchez
Bogotá, D.C., Colombia

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

En la Notaría Cincuenta de [illegible] cotejó la presente fotocopia con el original que se [illegible]

12 OCT. 2[illegible]2

NOTARIO CUA[illegible]E)
Marby Fernando A[illegible]
Bogotá, D.C., [illegible]

488

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.



Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900386

FECHA: 05 – 24 – 05

489

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.

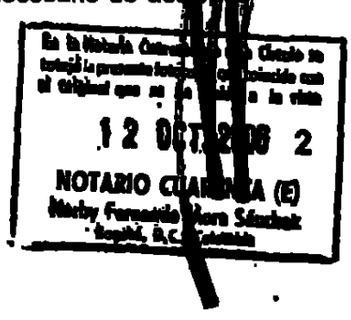
CERTIFICA POR LA PRESENTE:

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 común de la Corporación será toda de una misma clase.

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.



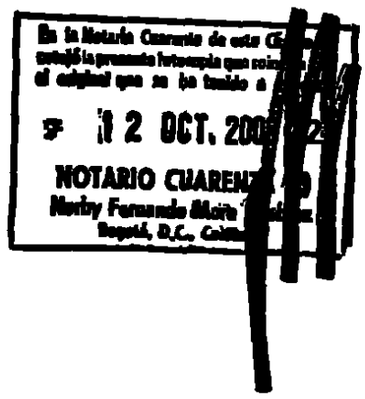
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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.



491

PETITORIO

AS: 102.470

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-119719- -00000-0000
Fec: 2006-11-27 16:59:34 Dep: 2020 NUECREACIONES
Tra: 11 PATENTEMII
Eve: 378 FASENACIONALI
Act: 411 PRESENTACION Folios: 491

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

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, CA 94080-4990, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail	IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número ____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Carrera 7 # 71 – 21 Torre B – Piso 4 Teléfono: 744-2200 Fax: 744-2700 E-mail:	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número 80.410.337 TP 57492
4 INVENTOR (ES) (72)	Nombre: Allen J. EBENS, JR., Frederic S. JACOBSON, Paul POLAKIS, Ralph H. SCHWALL, Mark X. SLIWKOWSKI, Susan D. SPENCER Dirección: 1932 Arroyo Ave, San Carlos, CA 94070 (US), 830 Shattuck Avenue, Berkeley, CA 94707 (US), 1449 Cortez Avenue, Burlingame, CA 94010 (US), 400 Griffin Avenue, Pacifica, CA 94044 (US), 42 Oak Creek Lane, San Carlos, CA 94070 (US), 2395 Paradise Drive, Tiburon, CA 94920 (US) (Respectivamente) Nacionalidad o Domicilio: Estados Unidos de América (Respectivamente)	

5 PCT (86)
Solicitud Internacional No. PCT/US2005/018829 Fecha 31-05-2005
Publicación Internacional No. WO 2005/117986 A2 Fecha 15-12-2005

6 Título (54): CONJUGADOS DE ANTICUERPO-MEDICAMENTO Y MÉTODOS

7 Clasificación Internacional (51) ____

8 Prioridad SI ☒ NO ☐	(33) País de Origen	(32) Fecha	(31) Número de Solicitud
	US	01-06-2004	60/576.517
	US	05-10-2004	60/616.098

9 Comprobante de pago No. 06-76.641 **Fecha** 27-11-2006

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

2020-F06 (01-11-15)

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-119719- -00000-0000
Fec: 2006-11-27 16:59:34 Dep. 2020 NUECREACIONES
Tra. 11 PATENTE(YII
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Comercio
ENDENCIA

491

REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [X] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [X]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- Descripción de la invención [X]
- Dibujos de ser estos pertinentes [ ]
- Comprobante de pago de las tasas establecidas [X]

COMPLETA [ ] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Firma Responsable

Fecha 27 NOV. 2006

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

4
493

Bogotá,
2020

Doctor(a)
CADENA SARMIENTO JUAN PABLO
Apoderado(a) y/o Representante de
GENENTECH, INC.

REFERENCIA	Radicación No.	06 119719
	Solicitud internacional	PCT/US2005/018829
	Fecha inicio fase nacional	01/01/2007
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	1874

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. El solicitante debe presentar resumen para efectos posteriores de publicación.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMENZA ESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 09 FEB. 2007
lerazo