SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCION

ENTRADA EN FASE NACIONAL

21. **EXPEDIENTE No.** 07-38013

54. **TITULO** FORMULACION DE ANTICUERPO EN AMORTIGUADOR DE ACETATO DE HISTIDINA

51. **CLASIFICACION INTERNACIONAL**

71. **SOLICITANTE** GENENTECH, INC.

DOMICILIO SOUTH SAN FRANCISCO, CA 94080-4990, ESTADOS UNIDOS

74. **APODERADO** JUAN PABLO CADENA SARMIENTO

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

F.N. 20-05-2007

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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, US 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:** James D. ANDYA, Shiang C. GWEE, Jun LIU, Ye SHEN **Dirección:** 227 Richmond. Drive, Apt. D, Millbrae, CA 94030 (US); 565 Crespi Drive, Pacifica, CA 94044 (US); 1106 Glacier Avenue, Pacifica, CA 94044 (US); 386 Faxon Avenue, San Francisco, CA 94112, US (Respectivamente) **Nacionalidad o Domicilio:** Estados Unidos de América (Respectivamente)	

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6 Título (54): FORMULACIÓN DE ANTICUERPO EN AMORTIGUADOR DE ACETATO DE HISTIDINA

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

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2

10 ANEXOS

- [x] Comprobante de pago de la tasa de presentación de la solicitud. $482.000
- [x] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [x] Poderes, si fuere el caso. **Fotocopia Autenticada**
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [ ] Declaraciones
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar efectuado por la IPEA.
- [ ] Traducción del examen preliminar efectuado por la IPEA.
- [ ] De ser el caso, copia del contrato de acceso.
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- [ ] De ser el caso, certificado de depósito del material biológico.
- [ ] Arte final 12x12.
- [ ] De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- [x] Otros: Listado de Secuencias
  Tarjeta de Propietarios
  Documentos PCT

11 FIGURA CARACTERÍSTICA:

12 Solicito la concesión de la patente,

NOMBRE: JUAN PABLO CADENA SARMIENTO

FIRMA:

C.C. 80.410.337 de Usaquén TP 57492 del C.S.J.

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CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	482,000.00
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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PATENTE [X] PCT/US2005/037471 MODELO DE UTILIDAD [ ] DISEÑO INDUSTRIAL [ ]

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

DOMICILIO SOUTH SAN FRANCISCO, CA 94080-4990, ESTADOS UNIDOS DE AMERICA

APODERADO JUAN PABLO CADENA SARMIENTO

TITULO FORMULACION DE ANTICUERPO EN AMORTIGUADOR DE ACETATO DE HISTIDINA

CLASIFICACION

EXPEDIENTE No. FECHA DE SOLICITUD ROLLO MICROFILM

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PRORROGA CAMBIO DE NOMBRE

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(71) Applicant *(for all designated States except US)*: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ANDYA, James, D. [US/US]; 227 Richmond Drive, Apt. D, Millbrae, CA 94030 (US). GWEE, Shiang, C. [US/US]; 565 Crespi Drive, Pacifica, CA 94044 (US). LIU, Jun [US/US]; 1108 Glacier Avenue, Pacifica, CA 94044 (US). SHEN, Ye [US/US]; 386 Faxon Avenue, San Francisco, CA 94112 (US).

(74) Agents: LEE, Wendy, M. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).

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(54) Title: ANTIBODY FORMULATION IN HISTIDINE-ACETATE BUFFER

(57) Abstract: The present application describes antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

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WO 2006/044908 A2

(54) Title: ANTIBODY FORMULATIONS

(57) Abstract: The present application describes antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

ANTIBODY FORMULATIONS

This is a non-provisional application filed under 37 CFR 1.53(b) claiming priority to provisional application 60/620,413 filed October 20, 2004, the contents of which are incorporated herein by reference

Field of the Invention

The present invention concerns antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

Background of the Invention

In the past ten years, advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications using recombinant DNA techniques. Because proteins are larger and more complex than traditional organic and inorganic drugs (*i.e.* possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (*i.e.* any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (*i.e.* changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation, deamidation and oxidation. Cleland *et al. Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993).

Antibody Formulations

Included in the proteins used for pharmaceutical applications are antibodies. An example of an antibody useful for therapy is an antibody which binds to the HER2 antigen, such as Pertuzumab.

US Patent No. 6,339,142 describes a HER2 antibody composition comprising a mixture of anti-HER2 antibody and one or more acidic variants thereof, wherein the amount of the acidic variant(s) is less than about 25%. Trastuzumab is an exemplified HER2 antibody.

US Patent Nos. 6,267,958 and 6,685,940 (Andya *et al.*) describe lyophilized antibody formulations, including HER2 and IgE antibody formulations. WO97/04807 and US 2004/0197326A1 (Fick *et al.*) describe methods for treating allergic asthma with an IgE antibody. WO99/01556 (Lowman *et al.*) relates to IgE antibody with aspartyl residues prone to isomerization, and improved variants thereof. US 2002/0045571 (Liu *et al.*) provides reduced viscosity concentrated protein formulations, exemplified by humanized IgE antibody formulations, rhuMAb E25 and E26. WO 02/096457 and US 2004/0170623 (Arvinte *et al.*) describes stable liquid formulations comprising anti-IgE antibody E25. See, also, US 2004/0197324 A1 (Liu and Shire) concerning high concentration anti-IgE formulation.

US Patent No. 6,171,586 (Lam *et al.*) describes stable aqueous antibody formulations. A F(ab')2 rhuMAb CD18 antibody was formulated in sodium acetate and histidine-HCl buffers. The preferred formulation for rhuMAb CD18 was 10mM sodium acetate, 8% trehalose, 0.01% TWEEN 20™, pH 5.0. Acetate (pH 5.0)

formulations of rhuMAb CD20 stored at 40° for one month demonstrated greater stability than those samples formulated in histidine (pH 5.0 or 6.0).

US 2003/0190316 (Kakuta *et al.*) concerns formulated antibody hPM-1, a humanized IL-6 receptor antibody. Monomer loss was the greatest in sodium citrate (pH 6.7), followed by sodium phosphate (pH 6.8), Tris-HCl (pH 7.2), histidine-HCl (pH 7.2) and glycine (pH 7.6) in descending order. The effect of phosphate-Na (pH 6.5), phosphate-His (pH 6.0 or 6.5), His-HCl (pH 6.5), and phosphate-Na (pH 6.0) on the stability of hPM-1 was assessed.

WO2004/071439 (Burke *et al.*) state that impurities arose in a natalizumab (anti-alpha4 integrin humanized monoclonal antibody) formulation from the degradation of polysorbate 80, apparently through an oxidation reaction involving metal ions and hisitidine. Thus, a phosphate buffer was selected.

WO 2000/066160 (English language counterpart EP 1 174 148A1) (Okada *et al.*) refers to a formulation of a humanized C4G1 antibody which binds to a fibrinogen receptor of a human platelet membrane glycoprotein GPIIb/IIIa, in a sodium phosphate or sodium citrate buffer.

WO2004/019861 (Johnson *et al.*) concerns CDP870, a pegylated anti-TNFα Fab fragment, formulated at 200mg/ml in 50mM sodium acetate (pH 5.5) and 125mM sodium chloride.

WO2004/004639 (Nesta, P.) refers to a formulation for huC242-DM1, a tumor-activated immunotoxin, in a 50mM succinic acid buffer (pH 6.0) and sucrose (5%w/v).

WO03/039485 (Kaisheva *et al.*) found that Daclizumab (a humanized IL-2 receptor antibody) had the highest stability in sodium succinate buffer at pH 6.0, and rapidly lost potency in histidine as the buffer oxidized.

WO 2004/001007 concerns a CD80 monoclonal antibody in a histidine HCl, sodium acetate or sodium citrate buffer.

US Patent No. 6,252,055 (Ralton, J.) refers to anti-CD4 and anti-CD23 antibodies formulated in maleate, succinate, sodium acetate or phosphate buffers, with phosphate being identified as the preferred buffer.

US Patent No. 5,608,038 (Eibl *et al.*) refers to highly concentrated polyclonal immunoglobulin preparations with immunoglobulin, glucose or sucrose, and sodium chloride therein.

WO03/015894 (Oliver *et al.*) refers to an aqueous formulation of 100mg/mL SYNAGIS®, 25mM histidine-HCl, 1.6mM glycine, pH 6.0, and a lyophilized SYNAGIS® which when formulated (before lyophilization) contains 25mM histidine, 1.6mM glycine and 3% w/v mannitol at pH 6.0.

US 2004/0191243 A1 (Chen *et al.*) reports formulation of ABX-IL8, a human IgG2 antibody.

US 2003/0113316 A1 (Kaisheva *et al.*) refers to a lyophilized anti-IL2 receptor antibody formulation.

HER2 Antibodies

The HER family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, or HER1), HER2 (ErbB2 or $p185^{neu}$), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

EGFR, encoded by the *erb*B1 gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF-α), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway. Baselga and Mendelsohn *Pharmac. Ther.* 64:127-154 (1994). Monoclonal antibodies directed against the EGFR or its ligands, TGF-α and EGF, have been evaluated as therapeutic agents

In the treatment of such malignancies. See, *e.g.*, Baselga and Mendelsohn., *supra*; Masui *et al. Cancer Research* 44:1002-1007 (1984); and Wu *et al. J. Clin. Invest.* 95:1897-1905 (1995).

The second member of the HER family, $p185^{neu}$, was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the *neu* proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homolog of *neu* is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon *et al.*, *Science*, 235:177-182 (1987); Slamon *et al.*, *Science*, 244:707-712 (1989); and US Pat No. 4,968,603). To date, no point mutation analogous to that in the *neu* proto-oncogene has been reported for human tumors. Overexpression of HER2 (frequently but not uniformly due to gene amplification) has also been observed in other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. See, among others, King *et al.*, *Science*, 229:974 (1985); Yokota *et al.*, *Lancet*: 1:765-767 (1986); Fukushige *et al.*, *Mol Cell Biol.*, 6:955-958 (1986); Guerin *et al.*, *Oncogene Res.*, 3:21-31 (1988); Cohen *et al.*, *Oncogene*, 4:81-88 (1989); Yonemura *et al.*, *Cancer Res.*, 51:1034 (1991); Borst *et al.*, *Gynecol. Oncol.*, 38:364 (1990); Weiner *et al.*, *Cancer Res.*, 50:421-425 (1990); Kern *et al.*, *Cancer Res.*, 50:5184 (1990); Park *et al.*, *Cancer Res.*, 49:6605 (1989); Zhau *et al.*, *Mol. Carcinog.*, 3:254-257 (1990); Aasland *et al. Br. J. Cancer* 57:358-363 (1988); Williams *et al. Pathobiology* 59:46-52 (1991); and McCann *et al.*, *Cancer*, 65:88-92 (1990). HER2 may be overexpressed in prostate cancer (Gu *et al. Cancer Lett.* 99:185-9 (1996); Ross *et al. Hum. Pathol.* 28:827-33 (1997); Ross *et al. Cancer* 79:2162-70 (1997); and Sadasivan *et al. J. Urol.* 150:126-31 (1993)).

Antibodies directed against the rat $p185^{neu}$ and human HER2 protein products have been described. Drebin and colleagues have raised antibodies against the rat *neu* gene product, $p185^{neu}$. See, for example, Drebin *et al., Cell* 41:695-706 (1985); Myers *et al., Meth. Enzym.* 198:277-290 (1991); and WO94/22478. Drebin *et al. Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions of $p185^{neu}$ result in synergistic anti-tumor effects on *neu*-transformed NIH-3T3 cells implanted into nude mice. See also U.S. Patent 5,824,311 issued October 20, 1998.

Hudziak *et al.*, *Mol. Cell. Biol.* 9(3):1165-1172 (1989) describe the generation of a panel of HER2 antibodies which were characterized using the human breast tumor cell line SK-BR-3. Relative cell proliferation of the SK-BR-3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, 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 HER2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also U.S. Patent No. 5,677,171 issued October 14, 1997. The HER2 antibodies discussed in Hudziak *et al.* are further characterized in Fendly *et al. Cancer Research* 50:1550-1558 (1990); Kotts *et al. In Vitro* 26(3):59A (1990); Sarup *et al. Growth Regulation* 1:72-82 (1991); Shepard *et al. J. Clin. Immunol.* 11(3):117-127 (1991); Kumar *et al. Mol. Cell. Biol.* 11(2):979-986 (1991); Lewis *et al. Cancer Immunol. Immunother.* 37:255-263 (1993); Pietras *et al. Oncogene* 9:1829-1838 (1994); Vitetta *et al. Cancer Research* 54:5301-5309 (1994); Sliwkowski *et al. J. Biol. Chem.* 269(20):14661-14665 (1994); Scott *et al. J. Biol. Chem.* 266:14300-5 (1991); D'souza *et al. Proc. Natl. Acad. Sci.* 91:7202-7206 (1994); Lewis *et al. Cancer Research* 56:1457-1465 (1996); and Schaefer *et al. Oncogene* 15:1385-1394 (1997).

A recombinant humanized version of the murine HER2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2, Trastuzumab or HERCEPTIN®; U.S. Patent No. 5,821,337) is clinically active in patients with HER2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 (1996)). Trastuzumab received marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

Other HER2 antibodies with various properties have been described in Tagliabue *et al. Int. J. Cancer* 47:933-937 (1991); McKenzie *et al. Oncogene* 4:543-548 (1989); Maier *et al. Cancer Res.* 51:5361-5369 (1991); Bacus *et al. Molecular Carcinogenesis* 3:350-362 (1990); Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991); Bacus *et al. Cancer Research* 52:2580-2589 (1992); Xu *et al. Int. J. Cancer* 53:401-408 (1993); WO94/00136; Kasprzyk *et al. Cancer Research* 52:2771-2776 (1992);Hancock *et al. Cancer Res.* 51:4575-4580 (1991); Shawver *et al. Cancer Res.* 54:1367-1373 (1994); Arteaga *et al. Cancer Res.* 54:3758-3765 (1994); Harwerth *et al. J. Biol. Chem.* 267:15160-15167 (1992); U.S. Patent No. 5,783,186; and Klapper *et al. Oncogene* 14:2099-2109 (1997).

Homology screening has resulted in the identification of two other HER receptor family members; HER3 (US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989)) and HER4 (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)). Both of these receptors display increased expression on at least some breast cancer cell lines.

The HER receptors are generally found in various combinations in cells and heterodimerization is thought to increase the diversity of cellular responses to a variety of HER ligands (Earp *et al. Breast Cancer Research and Treatment* 35: 115-132 (1995)). EGFR is bound by six different ligands; epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin, heparin binding epidermal growth factor (HB-EGF), betacellulin and epiregulin (Groenen *et al. Growth Factors* 11:235-257 (1994)). A family of heregulin proteins resulting from alternative splicing of a single gene are ligands for HER3 and HER4. The heregulin family includes alpha, beta and gamma heregulins (Holmes *et al.*, *Science*, 256:1205-1210 (1992); U.S. Patent No. 5,641,869; and Schaefer *et al. Oncogene* 15:1385-1394 (1997)); neu differentiation factors (NDFs), glial growth factors (GGFs); acetylcholine receptor inducing activity (ARIA); and sensory and motor neuron derived factor (SMDF). For a review, see Groenen *et al. Growth Factors* 11:235-257 (1994); Lemke, G. *Molec. & Cell. Neurosci.* 7:247-262 (1996) and Lee *et al. Pharm. Rev.* 47:51-85 (1995). Recently three additional HER ligands were identified; neuregulin-2 (NRG-2) which is reported to bind either HER3 or HER4 (Chang *et al. Nature* 387 509-512 (1997); and Carraway *et al Nature* 387:512-516 (1997)); neuregulin-3 which binds HER4 (Zhang *et al. PNAS* (USA) 94(18):9562-7 (1997)); and neuregulin-4 which binds HER4 (Harari *et al. Oncogene* 18:2681-89 (1999)) HB-EGF, betacellulin and epiregulin also bind to HER4.

While EGF and TGFα do not bind HER2, EGF stimulates EGFR and HER2 to form a heterodimer, which activates EGFR and results in transphosphorylation of HER2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the HER2 tyrosine kinase. See Earp *et al.*, *supra*. Likewise, when HER3 is co-expressed with HER2, an active signaling complex is formed and antibodies directed against HER2 are capable of disrupting this complex (Sliwkowski *et al.*, *J. Biol. Chem.*, 269(20):14661-14665 (1994)). Additionally, the affinity of HER3 for heregulin (HRG) is increased to a higher affinity state when co-expressed

with HER2. See also, Levi *et al.*, *Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al.*, *Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); and Lewis *et al.*, *Cancer Res.*, 56:1457-1465 (1996) with respect to the HER2-HER3 protein complex. HER4, like HER3, forms an active signaling complex with HER2 (Carraway and Cantley, *Cell* 78:5-8 (1994)).

To target the HER signaling pathway, rhuMAb 2C4 (Pertuzumab, OMNITARG™) was developed as a humanized antibody that inhibits the dimerization of HER2 with other HER receptors, thereby inhibiting ligand-driven phosphorylation and activation, and downstream activation of the RAS and AKT pathways. In a phase I trial of Pertuzumab as a single agent for treating solid tumors, 3 subjects with advanced ovarian cancer were treated with Pertuzumab. One had a durable partial response, and an additional subject had stable disease for 15 weeks Agus *et al. Proc Am Soc Clin Oncol* 22: 192, Abstract 771 (2003).

DR5 Antibodies

Various ligands and receptors belonging to the tumor necrosis factor (TNF) superfamily have been identified in the art. Included among such ligands are tumor necrosis factor-alpha ("TNF-alpha"), tumor necrosis factor-beta ("TNF-beta" or "lymphotoxin-alpha"), lymphotoxin-beta ("LT-beta"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, LIGHT, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as Apo2L or TRAIL), Apo-3 ligand (also referred to as TWEAK), APRIL, OPG ligand (also referred to as RANK ligand, ODF, or TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK) (See, *e.g.*, Ashkenazi, *Nature Review*, 2:420-430 (2002); Ashkenazi and Dixit, *Science*, 281:1305-1308 (1998); Ashkenazi and Dixit, *Curr. Opin. Cell Biol.*, 11:255-260 (2000); Golstein, *Curr. Biol.*, 7:750-753 (1997) Wallach, *Cytokine Reference*, Academic Press, 2000, pages 377-411; Locksley *et al.*, *Cell*, 104:487-501 (2001); Gruss and Dower, *Blood*, 85:3378-3404 (1995); Schmid *et al.*, *Proc. Natl. Acad. Sci.*, 83:1881 (1986); Dealtry *et al.*, *Eur. J. Immunol.*, 17:689 (1987); Pitti *et al.*, *J. Biol. Chem.*, 271:12687-12690 (1996); Wiley *et al.*, *Immunity*, 3:673-682 (1995); *Browning et al.*, *Cell*, 72:847-856 (1993); Armitage *et al. Nature*, 357:80-82 (1992), WO 97/01633 published January 16, 1997; WO 97/25428 published July 17, 1997; Marsters *et al.*, *Curr. Biol.*, 8:525-528 (1998); Chicheportiche *et al.*, *Biol. Chem.*, 272:32401-32410 (1997); Hahne *et al.*, *J. Exp. Med.*, 188:1185-1190 (1998); WO98/28426 published July 2, 1998; WO98/46751 published October 22, 1998; WO98/18921 published May 7, 1998; Moore *et al.*, *Science*, 285:260-263 (1999); Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); Schneider *et al.*, *J. Exp. Med.*, 189:1747-1756 (1999); Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274:15978-15981 (1999)).

Induction of various cellular responses mediated by such TNF family ligands is typically initiated by their binding to specific cell receptors. Some, but not all, TNF family ligands bind to, and induce various biological activity through, cell surface "death receptors" to activate caspases, or enzymes that carry out the cell death or apoptosis pathway (Salvesen *et al.*, *Cell*, 91:443-446 (1997)). Included among the members of the TNF receptor superfamily identified to date are TNFR1, TNFR2, TACI, GITR,, CD27, OX-40, CD30, CD40, HVEM, Fas (also referred to as Apo-1 or CD95), DR4 (also referred to as TRAIL-R1), DR5 (also referred to as Apo-2 or TRAIL-R2), DcR1, DcR2, osteoprotegerin (OPG), RANK and Apo-3 (also referred to as DR3 or TRAMP) .

Most of these TNF receptor family members share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions, while others are found naturally as soluble proteins lacking a transmembrane and intracellular domain. The extracellular portion of typical TNFRs contains

a repetitive amino acid sequence pattern of multiple cysteine-rich domains (CRDs), starting from the NH_2-terminus.

The ligand referred to as Apo-2L or TRAIL was identified several years ago as a member of the TNF family of cytokines. (see, *e.g.*, Wiley *et al., Immunity*, 3:673-682 (1995); Pitti *et al., J. Biol. Chem.*, 271:12697-12690 (1996); WO 97/01633; WO 97/25428; US Patent 5,763,223 issued June 9, 1998; US Patent 6,284,236 issued September 4, 2001). The full-length native sequence human Apo2L/TRAIL polypeptide is a 281 amino acid long, Type II transmembrane protein. Some cells can produce a natural soluble form of the polypeptide, through enzymatic cleavage of the polypeptide's extracellular region (Mariani *et al., J. Cell. Biol.*, 137:221-229 (1997)). Crystallographic studies of soluble forms of Apo2L/TRAIL reveal a homotrimeric structure similar to the structures of TNF and other related proteins (Hymowitz *et al., Molec. Cell*, 4:563-571 (1999); Cha *et al., Immunity*, 11:253-261 (1999); Mongkolsapaya *et al., Nature Structural Biology*, 6:1048 (1999); Hymowitz *et al., Biochemistry*, 39:633-644 (2000)). Apo2L/TRAIL, unlike other TNF family members however, was found to have a unique structural feature in that three cysteine residues (at position 230 of each subunit in the homotrimer) together coordinate a zinc atom, and that the zinc binding is important for trimer stability and biological activity. (Hymowitz *et al., supra*; Bodmer *et al., J. Biol. Chem.*, 275:20632-20637 (2000)).

It has been reported in the literature that Apo2L/TRAIL may play a role in immune system modulation, including autoimmune diseases such as rheumatoid arthritis (see, *e.g.*, Thomas *et al., J. Immunol.*, 161:2195-2200 (1998); Johnsen *et al., Cytokine*, 11:664-672 (1999); Griffith *et al., J. Exp. Med.*, 189:1343-1353 (1999); Song *et al., J. Exp. Med.*, 191:1095-1103 (2000)).

Soluble forms of Apo2L/TRAIL have also been reported to induce apoptosis in a variety of cancer cells, including colon, lung, breast, prostate, bladder, kidney, ovarian and brain tumors, as well as melanoma, leukemia, and multiple myeloma (see, *e.g.*, Wiley *et al., supra*; Pitti *et al., supra*; US Patent 6,030,945 issued February 29, 2000; US Patent 6,746,668 issued June 8, 2004; Rieger *et al., FEBS Letters*, 427:124-128 (1998); Ashkenazi *et al., J. Clin. Invest.*, 104:155-162 (1999); Walczak *et al., Nature Med.*, 5:157-163 (1999); Keane *et al., Cancer Research*, 59:734-741 (1999); Mizutani *et al., Clin. Cancer Res.*, 5:2605-2612 (1999); Gazitt, *Leukemia*, 13:1817-1824 (1999); Yu *et al., Cancer Res.*, 60:2384-2389 (2000); Chinnaiyan *et al., Proc. Natl. Acad. Sci.*, 97:1754-1759 (2000)). *In vivo* studies in murine tumor models further suggest that Apo2L/TRAIL, alone or in combination with chemotherapy or radiation therapy, can exert substantial anti-tumor effects (see, *e.g.*, Ashkenazi *et al., supra*; Walczak *et al., supra*; Gliniak *et al., Cancer Res.*, 59:6153-6158 (1999); Chinnaiyan *et al., supra*; Roth *et al., Biochem. Biophys. Res. Comm.*, 265:1999 (1999); PCT Application US/00/15512; PCT Application US/01/23691). In contrast to many types of cancer cells, most normal human cell types appear to be resistant to apoptosis induction by certain recombinant forms of Apo2L/TRAIL (Ashkenazi *et al., supra;* Walzcak *et al., supra*). Jo *et al.* has reported that a polyhistidine-tagged soluble form of Apo2L/TRAIL induced apoptosis *in vitro* in normal isolated human, but not non-human, hepatocytes (Jo *et al., Nature Med.*, 6:564-567 (2000); see also, Nagata, *Nature Med.*, 6:502-503 (2000)). It is believed that certain recombinant Apo2L/TRAIL preparations may vary in terms of biochemical properties and biological activities on diseased versus normal cells, depending, for example, on the presence or absence of a tag molecule, zinc content, and % trimer content (See, Lawrence *et al., Nature Med.*, Letter to the Editor, 7:383-385 (2001); Qin *et al., Nature Med.*, Letter to the Editor, 7:385-386 (2001)).

Apo2L/TRAIL has been found to bind at least five different receptors. At least two of the receptors which bind Apo2L/TRAIL contain a functional, cytoplasmic death domain. One such receptor has been referred to as "DR4" (and alternatively as TR4 or TRAIL-R1) (Pan *et al., Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002).

Another such receptor for Apo2L/TRAIL has been referred to as DR5 (it has also been alternatively referred to as Apo-2; TRAIL-R or TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER) (see, *e.g.*, Sheridan *et al., Science*, 277:818-821 (1997), Pan *et al., Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton et al., Curr. Biol., 7:693-696 (1997); Walczak *et al., EMBO J.*, 16:5386-5387 (1997); Wu *et al., Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; IS 6,743,625 issued June 1, 2004). Like DR4, DR5 is reported to contain a cytoplasmic death domain and be capable of signaling apoptosis upon ligand binding (or upon binding a molecule, such as an agonist antibody, which mimics the activity of the ligand). The crystal structure of the complex formed between Apo-2L/TRAIL and DR5 is described in Hymowitz *et al., Molecular Cell*, 4:563-571 (1999).

Upon ligand binding, both DR4 and DR5 can trigger apoptosis independently by recruiting and activating the apoptosis initiator, caspase-8, through the death-domain-containing adaptor molecule referred to as FADD/Mort1 (Kischkel *et al., Immunity*, 12:611-620 (2000); Sprick *et al., Immunity*, 12:599-609 (2000); Bodmer *et al., Nature Cell Biol.*, 2:241-243 (2000)).

Apo2L/TRAIL has been reported to also bind those receptors referred to as DcR1, DcR2 and OPG, which believed to function as inhibitors, rather than transducers of signaling (see., *e.g.*, DcR1 (also referred to as TRID, LIT or TRAIL-R3) (Pan *et al., Science*, 276:111-113 (1997); Sheridan *et al., Science*, 277:818-821 (1997); McFarlane *et al., J. Biol. Chem.*, 272:25417-25420 (1997); Schneider *et al., FEBS Letters*, 416:329-334 (1997); Degli-Esposti *et al., J. Exp. Med.*, 186:1165-1170 (1997); and Mongkolsapaya *et al., J. Immunol.*, 160:3-6 (1998)); DcR2 (also called TRUNDD or TRAIL-R4) (Marsters *et al.; Curr. Biol.*, 7:1003-1006 (1997); Pan *et al., FEBS Letters*, 424:41-45 (1998); Degli-Esposti *et al., Immunity*, 7:813-820 (1997)), and OPG. In contrast to DR4 and DR5, the DcR1 and DcR2 receptors do not signal apoptosis.

Certain antibodies which bind to the DR4 and/or DR5 receptors have been reported in the literature. For example, anti-DR4 antibodies directed to the DR4 receptor and having agonistic or apoptotic activity in certain mammalian cells are described in, *e.g.*, WO 99/37684 published July 29, 1999; WO 00/73349 published July 12, 2000; WO 03/066661 published August 14, 2003. See, also, *e.g.*, Griffith *et al., J. Immunol.*, 162:2597-2605 (1999); Chuntharapai *et al., J. Immunol.*, 166:4891-4898 (2001); WO 02/097033 published December 2, 2002; WO 03/042367 published May 22, 2003; WO 03/038043 published May 8, 2003; WO 03/037913 published

May 8, 2003. Certain anti-DR5 antibodies have likewise been described, see, *e.g.*, WO 98/51793 published November 8, 1998; Griffith *et al.*, *J. Immunol.*, 162:2597-2605 (1999); Ichikawa *et al.*, *Nature Med.*, 7:954-960 (2001); Hylander *et al.*, "An Antibody to DR5 (TRAIL-Receptor 2) Suppresses the Growth of Patient Derived Gastrointestinal Tumors Grown in SCID mice", Abstract, 2d International Congress on Monoclonal Antibodies in Cancers, Aug. 29-Sept. 1, 2002, Banff, Alberta, Canada; WO 03/038043 published May 8, 2003; WO 03/037913 published May 8, 2003. In addition, certain antibodies having cross-reactivity to both DR4 and DR5 receptors have been described (see, *e.g.*, US patent 6,252,050 issued June 26, 2001).

Summary of the Invention

The invention herein relates, at least in part, to the identification of histidine-acetate, pH 5.5 to 6.5, as a particularly useful buffer for formulating monoclonal antibodies, especially full length IgG1 antibodies which are susceptible to deamidation and/or aggregation. The formulation retards degradation of the antibody product therein.

Thus, in a first aspect, the invention concerns a stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5. The monoclonal antibody preferably binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

In addition, the invention concerns a method of treating a disease or disorder in a subject comprising administering the formulation to a subject in an amount effective to treat the disease or disorder.

In another aspect, the invention concerns a pharmaceutical formulation comprising: (a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; (b) histidine-acetate buffer, pH 5.5 to 6.5; (c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and (d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

The invention also provides a method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5.

In yet a further aspect, the invention concerns a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant.

The invention also relates to a pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The invention also pertains to a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

In another aspect, the invention concerns a pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

In yet another aspect, the invention provides a method of treating cancer in a subject, comprising administering the pharmaceutical formulation to the subject in an amount effective to treat the cancer.

The invention also concerns a vial with a stopper pierceable by a syringe or a stainless steel tank comprising the formulation inside the vial or tank, optionally in frozen form.

Moreover, the invention provides a method of making a pharmaceutical formulation comprising: (a) preparing the monoclonal antibody formulation; and (b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

Brief Description of the Drawings

Figure 1 depicts Domains I-IV (SEQ ID Nos.19-22, respectively) of the extracellular domain of HER2.

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (V_L) (Fig. 2A) and variable heavy (V_H) (Fig. 2B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 1 and 2, respectively); V_L and V_H domains of humanized 2C4 version 574 (SEQ ID Nos. 3 and 4, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 5 and 6, respectively). Asterisks identify differences between humanized 2C4 version 574 and murine monoclonal antibody 2C4 or between humanized 2C4 version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

Figures 3A and 3B show the amino acid sequences of Pertuzumab light chain and heavy chain (SEQ ID Nos. 15 and 16, respectively). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figures 4A and 4B show the amino acid sequences of Pertuzumab light and heavy chain, each including an intact amino terminal signal peptide sequence (SEQ ID Nos. 17 and 18, respectively).

Figure 5 depicts, schematically, binding of 2C4 at the heterodimeric binding site of HER2, thereby preventing heterodimerization with activated EGFR or HER3.

Figure 6 depicts coupling of HER2/HER3 to the MAPK and Akt pathways.

Figure 7 compares activities of Trastuzumab and Pertuzumab.

Figure 8 depicts stability of Pertuzumab formulation by ion exchange (IEX) analyses.

Figure 9 shows stability of Pertuzumab formulation by size exclusion chromatography (SEC) analysis.

Figure 10 reflects physical stability Pertuzumab in different formulations.

Figure 11 is from an agitation study of Pertuzumab liquid formulations.

Figure 12 is from another agitation study of Pertuzumab liquid formulations.

Figure 13 is from a freeze-thawing study of Pertuzumab formulation.

Figures 14A and 14B show the amino acid sequences of Trastuzumab light chain (SEQ ID No. 13) and heavy chain (SEQ ID No. 14).

Figures 15A and 15B depict a variant Pertuzumab light chain sequence (SEQ ID No. 23) and a variant Pertuzumab heavy chain sequence (SEQ ID No. 24).

Figure 16A and 16B shows oligosaccharide structures commonly observed in IgG antibodies.

Figures 17A and 17B show the sequences of the light and heavy chains (SEQ ID Nos. 37-44) of specific anti-IgE antibodies E25, E26, HAE1 and Hu-901. In Fig. 17A, the variable light domain ends with the residues VEIK, residue 111. In Fig. 17B, the variable heavy domain ends with the residues VTVSS, around residue 120.

Figure 18A is a sequence alignment comparing the amino acid sequences of the variable light domain (V_L) of each of murine 2H7 (SEQ ID No. 25), humanized 2H7v16 variant (SEQ ID No. 26), and the human

kappa light chain subgroup I (SEQ ID No. 27). The CDRs of V_L of 2H7 and hu2H7v16 are as follows: CDR1 (SEQ ID No. 57), CDR2 (SEQ ID No. 58), and CDR3 (SEQ ID No. 59).

Figure 18B is a sequence alignment comparing the amino acid sequences of the variable heavy domain (V_H) of each of murine 2H7 (SEQ ID No. 28), humanized 2H7v16 variant (SEQ ID No. 29), and the human consensus sequence of the heavy chain subgroup III (SEQ ID No. 30). The CDRs of V_H of 2H7 and hu2H7v16 are as follows: CDR1 (SEQ ID No. 60), CDR2 (SEQ ID No. 61), and CDR3 (SEQ ID No. 62).

In Fig. 18A and Fig. 18B, the CDR1, CDR2 and CDR3 in each chain are enclosed within brackets, flanked by the framework regions, FR1-FR4, as indicated. 2H7 refers to murine 2H7 antibody. The asterisks in between two rows of sequences indicate the positions that are different between the two sequences. Residue numbering is according to Kabat *et al. Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), with insertions shown as a, b, c, d, and e.

Figure 19 depicts variable domain sequences of three different VEGF antibodies with SEQ ID Nos. 31-36.

Figure 20 shows size exclusion chromatography (SEC) elution profile of the following Apomab samples: (a) control and formulations prepared at (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 and (e) pH 7.0. The formulated samples were stored at 40°C for 2 months prior to the analysis.

Figure 21 depicts pH rate profile for the loss in Apomab antibody monomer during storage. Monomer kinetics by SEC was monitored during storage at 30°C and 40°C and the first-order rate constants were calculated.

Figure 22 provides ion exchange chromatography (IEC) elution profile of Apomab samples as follows: (a) control and formulations prepared at (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 and (e) pH 7.0. The formulated samples were stored at 40°C for 2 months prior to the analysis.

Figure 23 shows pH rate profile for the loss in IEC main peak during storage. Main peak kinetics by IEC was monitored during storage at 30°C and 40°C and the first-order rate constants were calculated.

Figure 24 shows the nucleotide sequence of human Apo-2 ligand cDNA (SEQ ID No. 45) and its derived amino acid sequence (SEQ ID No. 46). The "N" at nucleotide position 447 (in SEQ ID No. 45) is used to indicate the nucleotide base may be a "T" or "G".

Figures 25A and 25B show the 411 amino acid sequence of human DR5 receptor (SEQ ID No. 47) as published in WO 98/51793 on November 19, 1998, and the encoding nucleotide sequence (SEQ ID No. 48).

Figures 26A and 26B show the 440 amino acid sequence of human DR5 receptor (SEQ ID No. 49) and the encoding nucleotide sequence (SEQ ID No. 50), as also published in WO 98/35986 on August 20, 1998.

Figure 27 shows the Apomab 7.3 heavy chain amino acid sequence (SEQ ID No. 51).

Figure 28 shows the Apomab 7.3 light chain amino acid sequence (SEQ ID No.52).

Figures 29 show the alignment of 16H2 heavy chain (SEQ ID No. 53) and Apomab 7.3 heavy chain (SEQ ID No. 51) amino acid sequences.

Figure 30 shows the alignment of 16H2 light chain (SEQ ID No. 54) and Apomab 7.3 light chain (SEQ ID No. 52) amino acid sequences.

Figures 31A and 31B depict the variable heavy amino acid sequence (Fig. 31A; SEQ ID No. 55) and variable light amino acid sequence (Fig. 31B; SEQ ID No. 56) of Apomab 7.3. CDR residues are identified in bold.

Figure 32 shows an alignment of the mature 2H7v16 and 2H7v511 light chains (SEQ ID Nos. 63 and 64, respectively). Sequences shown with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Figure 33 shows an alignment of the the mature 2H7v16 and 2H7v511 heavy chains (SEQ ID Nos. 65 and 66, respectively). Sequences shown with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Detailed Description of the Preferred Embodiments

I. Definitions

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

Herein, a "frozen" formulation is one at a temperature below 0°C. Generally, the frozen formulation is not freeze-dried, nor is it subjected to prior, or subsequent, lyophilization. Preferably, the frozen formulation comprises frozen drug substance for storage (in stainless steel tank) or frozen drug product (in final vial configuration).

A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Preferably, the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993), for example. Stability can be measured at a selected temperature for a selected time period. Preferably, the formulation is stable at about 40°C for at least about 2-4 weeks, and/or stable at about 5°C and/or 15°C for at least 3 months, and/or stable at about -20°C for at least 3 months or at least 1 year. Furthermore, the formulation is preferably stable following freezing (to, *e.g.*, -70°C) and thawing of the formulation, for example following 1, 2 or 3 cycles of freezing and thawing. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (*e.g.* Asn deamidation), oxidation (*e.g.* Met oxidation), isomerization (*e.g.* Asp isomerization), clipping/hydrolysis/fragmentation (*e.g.* hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc.

A "deamidated" monoclonal antibody herein is one in which one or more asparagine residue thereof has been derivitized, *e.g.* to an aspartic acid or an iso-aspartic acid.

An antibody which is "susceptible to deamidation" is one comprising one or more residue which has been found to be prone to deamidate.

An antibody which is "susceptible to aggregation" is one which has been found to aggregate with other antibody molecule(s), especially upon freezing and/or agitation.

An antibody which is "susceptible to fragmentation" is one which has been found to be cleaved into two or more fragments, for example at a hinge region thereof.

By "reducing deamidation, aggregation, or fragmentation" is intended preventing or decreasing the amount of deamidation, aggregation, or fragmentation relative to the monoclonal antibody formulated at a different pH or in a different buffer.

Herein, "biological activity" of a monoclonal antibody refers to the ability of the antibody to bind to antigen and result in a measurable biological response which can be measured *in vitro* or *in vivo*. Such activity may be antagonistic (for example where the antibody is a HER2 antibody) or agonistic (for instance where the antibody binds DR5). In the case of Pertuzumab, in one embodiment, the biological activity refers to the ability of the formulated antibody to inhibit proliferation of the human breast cancer cell line MDA-MB-175-VII. Where the antibody is Apomab, the biological activity can refer, for example, to the ability of the formulated antibody to kill colon carcinoma, Colo205, cells.

By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffer of this invention preferably has a pH in the range from about 5.0 to about 7.0, preferably from about 5.5 to about 6.5, for example from about 5.8 to about 6.2, and most preferably has a pH of about 6.0. Examples of buffers that will control the pH in this range include acetate, succinate, succinate, gluconate, histidine, citrate, glycylglycine and other organic acid buffers. The preferred buffer herein is a histidine buffer.

A "histidine buffer" is a buffer comprising histidine ions. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, histidine sulfate. The preferred histidine buffer identified in the examples herein was found to be histidine acetate. In the preferred embodiment, the histidine acetate buffer is prepared by titrating L-histidine (free base, solid) with acetic acid (liquid). Preferably, the histidine buffer or histidine-acetate buffer is at pH 5.5 to 6.5, preferably pH 5.8 to 6.2.

A "saccharide" herein comprises the general composition $(CH_2O)_n$ and derivatives thereof, including monosaccharides, disaccharides, trisaccharides, polysaccharides, sugar alcohols, reducing sugars, nonreducing sugars, etc. Examples of saccharides herein include glucose, sucrose, trehalose, lactose, fructose, maltose, dextran, glycerin, dextran, erythritol, glycerol, arabitol, sylitol, sorbitol, mannitol, mellibiose, melezitose, raffinose, mannotriose, stachyose, maltose, lactulose, maltulose, glucitol, maltitol, lactitol, iso-maltulose, etc. The preferred saccharide herein is a nonreducing disaccharide, such as trehalose or sucrose.

Herein, a "surfactant" refers to a surface-active agent, preferably a nonionic surfactant. Examples of surfactants herein include polysorbate (for example, polysorbate 20 and, polysorbate 80); poloxamer (*e.g.*

poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.* lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, New Jersey); polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.* Pluronics, PF68 etc); etc. The preferred surfactant herein is polysorbate 20.

A "HER receptor" is a receptor protein tyrosine kinase which belongs to the HER receptor family and includes EGFR, HER2, HER3 and HER4 receptors and other members of this family to be identified in the future. The HER receptor will generally comprise an extracellular domain, which may bind an HER 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. Preferably the HER receptor is native sequence human HER receptor.

The extracellular domain of HER2 comprises four domains, Domain I (amino acid residues from about 1-195), Domain II (amino acid residues from about 196-320), Domain III (amino acid residues from about 321-488), and Domain IV (amino acid residues from about 489-632) (residue numbering without signal peptide). See Garrett *et al. Mol. Cell.* 11: 495-505 (2003), Cho *et al. Nature* 421: 756-760 (2003), Franklin *et al. Cancer Cell* 5:317-328 (2004), or Plowman *et al. Proc. Natl. Acad. Sci.* 90:1746-1750 (1993). See also Fig. 1 herein.

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

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba *et al.*, *PNAS (USA)* 82:6497-6501 (1985) and Yamamoto *et al. Nature* 319:230-234 (1986) (Genebank accession number X03363). The term "*erbB2*" refers to the gene encoding human ErbB2 and "*neu*" refers to the gene encoding rat p185^{neu}. Preferred HER2 is native sequence human HER2.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989).

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 WO99/19488, published April 22, 1999.

By "HER ligand" is meant a polypeptide which binds to and/or activates a HER receptor. The HER ligand of particular interest herein is a native sequence human HER ligand such as epidermal growth factor (EGF) (Savage *et al.*, *J. Biol. Chem.* 247:7612-7621 (1972)); transforming growth factor alpha (TGF-α) (Marquardt *et al.*, *Science* 223:1079-1082 (1984)); amphiregulin also known as schwanoma or keratinocyte autocrine growth factor (Shoyab *et al. Science* 243:1074-1076 (1989); Kimura *et al. Nature* 348:257-260 (1990); and Cook *et al. Mol. Cell. Biol.* 11:2547-2557 (1991)); betacellulin (Shing *et al.*, *Science* 259:1604-1607 (1993);

and Sasada *et al. Biochem. Biophys. Res. Commun.* 190:1173 (1993)); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama *et al., Science* 251:936-939 (1991)); epiregulin (Toyoda *et al., J. Biol. Chem.* 270:7495-7500 (1995); and Komurasaki *et al. Oncogene* 15:2841-2848 (1997)); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway *et al., Nature* 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang *et al., Proc. Natl. Acad. Sci.* 94:9562-9567 (1997)); neuregulin-4 (NRG-4) (Harari *et al. Oncogene* 18:2681-89 (1999)) or cripto (CR-1) (Kannan *et al. J. Biol. Chem.* 272(6):3330-3335 (1997)). HER ligands which bind EGFR include EGF, TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. HER ligands which bind HER3 include heregulins. HER ligands capable of binding HER4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins.

"Heregulin" (HRG) when used herein refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Patent No. 5,641,869 or Marchionni *et al., Nature*, 362:312-318 (1993). Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes *et al., Science*, 256:1205-1210 (1992); and U.S. Patent No. 5,641,869); *neu* differentiation factor (NDF) (Peles *et al. Cell* 69: 205-216 (1992)); acetylcholine receptor-inducing activity (ARIA) (Falls *et al. Cell* 72:801-815 (1993)); glial growth factors (GGFs) (Marchionni *et al., Nature*, 362:312-318 (1993)); sensory and motor neuron derived factor (SMDF) (Ho *et al. J. Biol. Chem.* 270:14523-14532 (1995)); γ-heregulin (Schaefer *et al. Oncogene* 15:1385-1394 (1997)). 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.* $HRG\beta1_{177-244}$).

A "HER dimer" herein is a noncovalently associated dimer comprising at least two different HER receptors. Such complexes may form when a cell expressing two or more HER receptors is exposed to an HER ligand and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski *et al., J. Biol. Chem.*, 269(20):14661-14665 (1994), for example. Examples of such HER dimers include EGFR-HER2, HER2-HER3 and HER3-HER4 heterodimers. Moreover, the HER dimer may comprise two or more HER2 receptors combined with a different HER receptor, such as HER3, HER4 or EGFR. Other proteins, such as a cytokine receptor subunit (*e.g.* gp130) may be associated with the dimer.

A "heterodimeric binding site" on HER2, refers to a region in the extracellular domain of HER2 that contacts, or interfaces with, a region in the extracellular domain of EGFR, HER3 or HER4 upon formation of a dimer therewith. The region is found in Domain II of HER2. Franklin *et al. Cancer Cell* 5:317-328 (2004).

"HER activation" or "HER2 activation" refers to activation, or phosphorylation, of any one or more HER receptors, or HER2 receptors. Generally, HER activation results in signal transduction (*e.g.* that caused by an intracellular kinase domain of a HER receptor phosphorylating tyrosine residues in the HER receptor or a substrate polypeptide). HER activation may be mediated by HER ligand binding to a HER dimer comprising the HER receptor of interest. HER ligand binding to a HER dimer may activate a kinase domain of one or more of the HER receptors in the dimer and thereby results in phosphorylation of tyrosine residues in one or more of the HER receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s), such as Akt or MAPK intracellular kinases.

The term "antibody" herein is used in the broadest sense and specifically covers full length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two full length antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

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 and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624--628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

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

"Antibody fragments" comprise a portion of a full length antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

A "full length antibody" is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variants thereof. Preferably, the full length antibody has one or more effector functions.

The term "main species antibody" herein refers to the antibody structure in a composition which is the quantitatively predominant antibody molecule in the composition. In one embodiment, the main species antibody is a HER2 antibody, such as an antibody that binds to Domain II of HER2, antibody that inhibits HER dimerization more effectively than Trastuzumab, and/or an antibody which binds to a heterodimeric binding site of HER2. The preferred embodiment herein of a main species HER2 antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 15 and 16 (Pertuzumab).

An "amino acid sequence variant" antibody herein is an antibody with an amino acid sequence which differs from a main species antibody. Ordinarily, amino acid sequence variants will possess at least about 70%

homology with the main species antibody, and preferably, they will be at least about 80%, more preferably at least about 90% homologous with the main species antibody. The amino acid sequence variants possess substitutions, deletions, and/or additions at certain positions within or adjacent to the amino acid sequence of the main species antibody. Examples of amino acid sequence variants herein include acidic variant (*e.g.* deamidated antibody variant), basic variant, the antibody with an amino-terminal leader extension (*e.g.* VHS-) on one or two light chains thereof, antibody with a C-terminal lysine residue on one or two heavy chains thereof, etc, and includes combinations of variations to the amino acid sequences of heavy and/or light chains. The antibody variant of particular interest herein is the antibody comprising an amino-terminal leader extension on one or two light chains thereof, optionally further comprising other amino acid sequence and/or glycosylation differences relative to the main species antibody.

A "therapeutic monoclonal antibody" is an antibody used for therapy of a human subject. Therapeutic monoclonal antibodies disclosed herein include: HER2 antibodies for cancer and various non-malignant diseases or disorders; CD20 or BR3 antibodies for therapy of B cell malignancies, autoimmune diseases, graft rejection, or blocking an immune response to a foreign antigen; IgE antibodies for therapy of an IgE-mediated disorder; DR5 or VEGF antibodies for cancer therapy.

A "glycosylation variant" antibody herein is an antibody with one or more carbohydrate moeities attached thereto which differ from one or more carbohydate moieties attached to a main species antibody. Examples of glycosylation variants herein include antibody with a G1 or G2 oligosaccharide structure, instead a G0 oligosaccharide structure, attached to an Fc region thereof, antibody with one or two carbohydrate moieties attached to one or two light chains thereof, antibody with no carbohydrate attached to one or two heavy chains of the antibody, etc, and combinations of glycosylation alterations.

Where the antibody has an Fc region, an oligosaccharide structure such as that shown in Fig. 16 herein may be attached to one or two heavy chains of the antibody, *e.g.* at residue 299 (298, Eu numbering of residues). For Pertuzumab, G0 was the predominant oligosaccharide structure, with other oligosaccharide structures such as G0-F, G-1, Man5, Man6, G1-1, G1(1-6), G1(1-3) and G2 being found in lesser amounts in the Pertuzumab composition.

Unless indicated otherwise, a "G1 oligosaccharide structure" herein includes G-1, G1-1, G1(1-6) and G1(1-3) structures.

An "amino-terminal leader extension" herein refers to one or more amino acid residues of the amino-terminal leader sequence that are present at the amino-terminus of any one or more heavy or light chains of an antibody. An exemplary amino-terminal leader extension comprises or consists of three amino acid residues, VHS, present on one or both light chains of an antibody variant.

"Homology" 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 homology. 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 "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent

cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor; BCR), etc.

Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different "classes". There are five major classes of full length 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.

"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, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be *assessed in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, *e.g.* from blood or PBMCs as described herein.

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

"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.*, *J. Immunol. Methods* 202:163 (1996), 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 (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) 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.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). 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.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) 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 *J. Mol. Biol.* 196:901-917 (1987)). "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 V_H-V_L 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" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). HER2 antibody scFv fragments are described in WO93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

"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.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

Humanized HER2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 or Trastuzumab (HERCEPTIN®) as described in Table 3 of U.S. Patent 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319) and humanized 2C4 antibodies as described herein.

For the purposes herein, "Trastuzumab," "HERCEPTIN®," and "huMAb4D5-8" refer to an antibody comprising the light and heavy chain amino acid sequences in SEQ ID NOS. 13 and 14, respectively.

Herein, "Pertuzumab," "rhuMAb 2C4," and "OMNITARG™" refer to an antibody comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectfully. Where Pertuzumab is a full length antibody, it preferably comprises the light chain and heavy chain amino acid sequences in SEQ ID NOS. 15 and 16, respectively.

A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

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

An "agonist antibody" is an antibody which binds to and activates a receptor. Generally, the receptor activation capability of the agonist antibody will be at least qualitatively similar (and may be essentially quantitatively similar) to a native agonist ligand of the receptor. An example of an agonist antibody is one which binds to a receptor in the TNF receptor superfamily, such as DR5, and induces apoptosis of cells expressing the TNF receptor (*e.g.* DR5). Assays for determining induction of apoptosis are described in WO98/51793 and WO99/37684, both of which are expressly incorporated herein by reference.

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. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably 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 sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, 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.

A HER2 antibody which "inhibits HER dimerization more effectively than Trastuzumab" is one which reduces or eliminates HER dimers more effectively (for example at least about 2-fold more effectively) than Trastuzumab. Preferably, such an antibody inhibits HER2 dimerization at least about as effectively as an antibody selected from the group consisting of murine monoclonal antibody 2C4, a Fab fragment of murine monoclonal antibody 2C4, Pertuzumab, and a Fab fragment of Pertuzumab. One can evaluate HER dimerization inhibition by studying HER dimers directly, or by evaluating HER activation, or downstream signaling, which results from HER dimerization, and/or by evaluating the antibody-HER2 binding site, etc. Assays for screening for antibodies with the ability to inhibit HER dimerization more effectively than Trastuzumab are described in Agus *et al. Cancer Cell* 2: 127-137 (2002) and WO01/00245 (Adams *et al.*). By way of example only, one may assay for inhibition of HER dimerization by assessing, for example, inhibition of HER dimer formation (see, *e.g.*, Fig. 1A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002); and WO01/00245); reduction in HER ligand activation of cells which express HER dimers (WO01/00245and Fig. 2A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); blocking of HER ligand binding to cells which express HER dimers (WO01/00245, and Fig. 2E of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); cell growth inhibition of cancer cells (*e.g.* MCF7, MDA-MD-134, ZR-75-1, MD-MB-175, T-47D cells) which express HER dimers in the presence (or absence) of HER ligand (WO01/00245and Figs. 3A-D of Agus *et al. Cancer Cell* 2: 127-137 (2002), for instance); inhibition of downstream signaling (for instance, inhibition of HRG-dependent AKT phosphorylation or inhibition of HRG- or TGFα- dependent MAPK phosphorylation) (see, WO01/00245, and Fig. 2C-D of Agus et al. *Cancer Cell* 2: 127-137 (2002), for example). One may also assess whether the antibody inhibits HER dimerization by studying the antibody-HER2 binding site, for instance, by evaluating a structure or model, such as a crystal structure, of the antibody bound to HER2 (See, for example, Franklin *et al. Cancer Cell* 5:317-328 (2004)).

The HER2 antibody may "inhibit HRG-dependent AKT phosphorylation" and/or inhibit "HRG- or TGFα-dependent MAPK phosphorylation" more effectively (for instance at least 2-fold more effectively) than Trastuzumab (see Agus *et al. Cancer Cell* 2: 127-137 (2002) and WO01/00245, by way of example).

The HER2 antibody may be one which does "not inhibit HER2 ectodomain cleavage" (Molina *et al. Cancer Res.* 61:4744-4749(2001).

A HER2 antibody that "binds to a heterodimeric binding site" of HER2, binds to residues in domain II (and optionally also binds to residues in other of the domains of the HER2 extracellular domain, such as domains I and III), and can sterically hinder, at least to some extent, formation of a HER2-EGFR, HER2-HER3, or HER2-HER4 heterodimer. Franklin *et al. Cancer Cell* 5:317-328 (2004) characterize the HER2-Pertuzumab crystal structure, deposited with the RCSB Protein Data Bank (ID Code IS78), illustrating an exemplary antibody that binds to the heterodimeric binding site of HER2.

An antibody that "binds to domain II" of HER2 binds to residues in domain II and optionally residues in other domain(s) of HER2, such as domains I and III. Preferably the antibody that binds to domain II binds to the junction between domains I, II and III of HER2.

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

agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

Examples of "growth inhibitory" antibodies are those which bind to HER2 and inhibit the growth of cancer cells overexpressing HER2. Preferred growth inhibitory HER2 antibodies inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, and preferably 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 (see U.S. Patent No. 5,677,171 issued October 14, 1997). The SK-BR-3 cell growth inhibition assay is described in more detail in that patent and hereinbelow. The preferred growth inhibitory antibody is a humanized variant of murine monoclonal antibody 4D5, *e.g.*, Trastuzumab.

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 expresses the antigen to which the antibody binds. Preferably the cell is a tumor cell. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay using cells that express an antigen to which the antibody binds. Examples of antibodies that induce apoptosis are HER2 antibodies 7C2 and 7F3, and certain DR5 antibodies.

The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2. Epitope 2C4 comprises residues from domain II in the extracellular domain of HER2. 2C4 and Pertuzumab bind to the extracellular domain of HER2 at the junction of domains I, II and III. Franklin *et al. Cancer Cell* 5:317-328 (2004).

The "epitope 4D5" is the region in the extracellular domain of HER2 to which the antibody 4D5 (ATCC CRL 10463) and Trastuzumab bind. This epitope is close to the transmembrane domain of HER2, and within Domain IV of HER2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 4D5 epitope of HER2 (*e.g.* any one or more residues in the region from about residue 529 to about residue 625, inclusive, of HER2).

The "epitope 7C2/7F3" is the region at the amino terminus, within Domain I, of the extracellular domain of HER2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on HER2 (*e.g.* any one or more of residues in the region from about residue 22 to about residue 53 of HER2).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disease as well as those in which the disease is to be prevented. Hence, the patient to be treated herein may have been diagnosed as having the disease or may be predisposed or susceptible to the disease.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma,and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, as well as head and neck cancer.

The term "effective amount" refers to an amount of a drug effective to a disease in the patient. Where the disease is cancer, the effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. The effective amount may extend progression free survival, result in an objective response (including a partial response, PR, or complete response, CR), increase overall survival time, and/or improve one or more symptoms of cancer.

A "HER2-expressing cancer" is one comprising cells which have HER2 protein present at their cell surface.

A cancer which "overexpresses" a HER receptor is one which has significantly higher levels of a HER receptor, such as HER2, 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. HER receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the HER protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of HER-encoding nucleic acid in the cell, *e.g.* via fluorescent *in situ*

hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study HER receptor overexpression by measuring shed antigen (*e.g.*, HER extracellular domain) in a biological fluid such as serum (see, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias *et al. J. Immunol. Methods* 132: 73-80 (1990)). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

Conversely, a cancer which "does not overexpress HER2 receptor" is one which does not express higher than normal levels of HER2 receptor compared to a noncancerous cell of the same tissue type.

A cancer which "overexpresses" a HER ligand is one which produces significantly higher levels of that ligand compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. Overexpression of the HER 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.

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.* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} 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 fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores),

aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), peglylated liposomal doxorubicin (CAELYX®), 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, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, 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; 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; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin, and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovovin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (*e.g.*, LURTOTECAN®); rmRH (*e.g.*, ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (Pfizer); perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin,

vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SHRMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrazole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors including vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, imidazole; lutenizing hormone-releasing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and tripterelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretionic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; testolactone; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

As used herein, the term "EGFR-targeted drug" refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO98/50433, Abgenix). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659,439A2, Merck Patent GmbH). Examples of small molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), CP-358774 or Erlotinib HCL (TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen).

A "tyrosine kinase inhibitor" is a molecule which inhibits to some extent tyrosine kinase activity of a tyrosine kinase such as a HER receptor. Examples of such inhibitors include the EGFR-targeted drugs noted in the preceding paragraph as well as small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda, dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 & EGFR-overexpressing cells, GW572016 (available from Glaxo) an oral HER2 and EGFR tyrosine kinase inhibitor, and PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibits Raf-1 signaling; non-HER targeted TK inhibitors such as Imatinib mesylate (Gleevac™) available from Glaxo; MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines;

pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules (*e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER 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); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: US Patent No. 5,804,396; WO99/09016 (American Cyanimid); WO98/43960 (American Cyanamid); WO97/38983 (Warner Lambert); WO99/06378 (Warner Lambert); WO99/06396 (Warner Lambert); WO96/30347 (Pfizer, Inc); WO96/33978 (Zeneca); WO96/3397 (Zeneca); and WO96/33980 (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. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF), such as Bevacizumab (AVASTIN®).

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 antibody which is formulated is preferably essentially pure and desirably essentially homogeneous (*i.e.* free from contaminating proteins etc). "Essentially pure" antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antibody that binds thereto. Exemplary B-cell surface markers include the

CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers (for descriptions, see The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Other B-cell surface markers include RP105, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCRR2, BR3, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest herein is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface marker herein is CD20 or BR3.

The "CD20" antigen, or "CD20," is an about 35-kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is present on both normal B cells as well as malignant B cells, but is not expressed on stem cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al.* *Proc. Natl. Acad. Sci.* (USA) 82:1766 (1985), for example.

Purely for the purposes herein, "humanized 2H7" refers to a humanized variant of the 2H7 antibody whose CDR sequences are disclosed in US Patent No. 5,500,362 (Figs. 5 and 6), expressly incorporated herein by reference. Examples of humanized 2H7 antibodies herein include the variants described in WO2004/056312, also expressly incorporated herein by reference, as well as other variants, including, but not limited to: 2H7v16, 2H7v31, 2H7v73, 2H7v75, 2H7v96, 2H7v114, 2H7v115, 2H7v116, 2H7v138, 2H7v477, 2H7v375, etc.

In one embodiment, the humanized 2H7 antibody comprises one, two, three, four, five or six of the following CDR sequences:

CDR L1 sequence RASSSVSYXH wherein X is M or L (SEQ ID No. 67), for example SEQ ID No. 57 (Fig. 18A),

CDR L2 sequence of SEQ ID No. 58 (Fig. 18A),

CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID No. 68), for example SEQ ID No. 59 (Fig. 18A),

CDR H1 sequence of SEQ ID No. 60 (Fig. 18B),

CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID No. 69), for example SEQ ID No. 61 (Fig. 18B), and

CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W or D, and the X at position 7 is S or R (SEQ ID No. 70), for example SEQ ID No. 62 (Fig. 18B).

The CDR sequences above are generally present within human variable light and variable heavy framework sequences, such as substantially the human consensus FR residues of human light chain kappa subgroup I ($V_LκI$), and substantially the human consensus FR residues of human heavy chain subgroup III (V_HIII). See also WO 2004/056312 (Lowman *et al.*).

The variable heavy region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3, including native sequence and variant constant regions.

In a preferred embodiment, such antibody comprises the variable heavy domain sequence of SEQ ID No. 29 (v16, as shown in Fig. 18B), optionally also comprising the variable light domain sequence of SEQ ID No. 26 (v16, as shown in Fig. 18A), which optionally comprises one or more amino acid substitution(s) at positions 56, 100, and/or 100a, *e.g.* D56A, N100A or N100Y, and/or S100aR in the variable heavy domain and

one or more amino acid substitution(s) at positions 32 and/or 92, *e.g.* M32L and/or S92A, in the variable light domain. Preferably, the antibody is an intact antibody comprising the light chain amino acid sequences of SEQ ID Nos. 63 or 64, and heavy chain amino acid sequences of SEQ ID No. 65, 66, 71 or 72.

A preferred humanized 2H7 antibody is ocrelizumab (Genentech).

The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC activity, such as one wherein the amino acid substitutions are at positions 298, 333, and 334, preferably S298A, E333A, and K334A, using Eu numbering of heavy chain residues. See also US Patent No. 6,737,056B1, Presta.

Any of these antibodies may comprise at least one substitution in the Fc region that improves FcRn binding or serum half-life, for example a substitution at heavy chain position 434, such as N434W. See also US Patent No. 6,737,056B1, Presta.

Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that increases CDC activity, for example, comprising at least a substitution at position 326, preferably K326A or K326W. See also US Patent No. 6,528,624B1 (Idusogie *et al.*).

Some preferred humanized 2H7 variants are those comprising the variable light domain of SEQ ID No. 26 and the variable heavy domain of SEQ ID No. 29, including those with or without substitutions in an Fc region (if present), and those comprising a variable heavy domain with alteration N100A; or D56A and N100A; or D56A, N100Y, and S100aR; in SEQ ID No. 29 and a variable light domain with alteration M32L; or S92A; or M32L and S92A; in SEQ ID No. 26.

M34 in the variable heavy chain of 2H7v16 has been identified as a potential source of antibody stability and is another potential candidate for substitution.

In a summary of some various preferred embodiments of the invention, the variable region of variants based on 2H7v16 comprise the amino acid sequences of v16 except at the positions of amino acid substitutions that are indicated in the Table below. Unless otherwise indicated, the 2H7 variants will have the same light chain as that of v16.

Exemplary Humanized 2H7 Antibody Variants

2H7 Version	Heavy chain (V_H) changes	Light chain (V_L) changes	Fc changes
16 for reference			-
31	-	-	S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N100A	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K334A, K322A
138	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
477	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
375	-	-	K334L
588	-	-	S298A, E333A, K334A, K326A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, E333A, K334A, K326A

One preferred humanized 2H7 comprises 2H7v16 variable light domain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID No. 26);

and 2H7v16 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID No. 29).

Where the humanized 2H7v16 antibody is an intact antibody, it may comprise the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID No. 63);

and the heavy chain amino acid sequence of SEQ ID No. 65 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 71).

Another preferred humanized 2H7 antibody comprises 2H7v511 variable light domain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID No. 73)

and 2H7v511 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS (SEQ ID No. 74).

Where the humanized 2H7v511 antibody is an intact antibody, it may comprise the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID No. 64)

and the heavy chain amino acid sequence of SEQ ID No. 66 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAA TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 72).

A "B-cell malignancy" herein includes non-Hodgkin's lymphoma (NHL), including low grade/follicular NHL, small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia; leukemia, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia and chronic myeloblastic leukemia; and other hematologic malignancies. Such malignancies may be treated with antibodies directed against B-cell surface markers, such as CD20.

The term "non-Hodgkin's lymphoma" or "NHL", as used herein, refers to a cancer of the lymphatic system other than Hodgkin's lymphomas. Hodgkin's lymphomas can generally be distinguished from non-Hodgkin's lymphomas by the presence of Reed-Sternberg cells in Hodgkin's lymphomas and the absence of said cells in non-Hodgkin's lymphomas. Examples of non-Hodgkin's lymphomas encompassed by the term as used herein include any that would be identified as such by one skilled in the art (*e.g.*, an oncologist or pathologist) in accordance with classification schemes known in the art, such as the Revised European-American Lymphoma (REAL) scheme as described in Color Atlas of Clinical Hematology, Third Edition; A. Victor Hoffbrand and John E. Pettit (eds.) (Harcourt Publishers Limited 2000) (see, in particular Fig. 11.57, 11.58 and/or 11.59). More specific examples include, but are not limited to, relapsed or refractory NHL, front line low grade NHL, Stage III/IV NHL, chemotherapy resistant NHL, precursor B lymphoblastic leukemia and/or lymphoma, small lymphocytic lymphoma, B cell chronic lymphocytic leukemia and/or prolymphocytic leukemia and/or small lymphocytic lymphoma, B-cell prolymphocytic lymphoma, immunocytoma and/or lymphoplasmacytic lymphoma, marginal zone B cell lymphoma, splenic marginal zone lymphoma, extranodal marginal zone - MALT lymphoma, nodal marginal zone lymphoma, hairy cell leukemia, plasmacytoma and/or plasma cell myeloma, low grade/follicular lymphoma, intermediate grade/follicular NHL, mantle cell lymphoma, follicle center lymphoma (follicular), intermediate grade diffuse NHL, diffuse large B-cell lymphoma, aggressive NHL (including aggressive front-line NHL and aggressive relapsed NHL), NHL relapsing after or refractory to autologous stem cell transplantation, primary mediastinal large B-cell lymphoma, primary effusion lymphoma, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, Burkitt's lymphoma, precursor (peripheral) T-cell lymphoblastic leukemia and/or lymphoma, adult T-cell lymphoma and/or leukemia, T cell chronic lymphocytic leukemia and/or prolymphacytic leukemia, large granular lymphocytic leukemia, mycosis fungoides and/or Sezary syndrome, extranodal natural killer/T-cell (nasal type) lymphoma, enteropathy type T-cell lymphoma, hepatosplenic T-cell lymphoma, subcutaneous panniculitis like T-cell lymphoma, skin (cutaneous) lymphomas, anaplastic large cell lymphoma, angiocentric lymphoma, intestinal T cell lymphoma, peripheral T-cell (not otherwise specified) lymphoma and angioimmunoblastic T-cell lymphoma.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis, juvenile-onset rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis, chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as progressive systemic sclerosis, inflammatory bowel disease (IBD) (for example, Crohn's

disease, ulcerative colitis, autoimmune inflammatory bowel disease), pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis or autoimmune uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN (membranous nephropathy), idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, allergic reaction, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, subacute cutaneous lupus erythematosus, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), multiple sclerosis (MS) such as spino-optical MS, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitis (including large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), CNS vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis , such as Churg-Strauss vasculitis or syndrome (CSS)), temporal arteritis, aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Behcet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Raynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's disease, immune complex nephritis, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP) and autoimmune or immune-mediated thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, autoimmune disease of the testis and ovary including autoimune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Addison's disease, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis, myasthenia gravis, cerebellar degeneration, limbic and/or brainstem encephalitis, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), primary biliary cirrhosis,

celiac sprue (gluten enteropathy), refractory sprue, dermatitis herpetiformis, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), or autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases, Dressler's syndrome, alopecia arcata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, or Fuch's cyolitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, and giant cell polymyalgia.

The "tumor necrosis factor receptor superfamily" or "TNF receptor superfamily" herein refers to receptor polypeptides bound by cytokines in the TNF family. Generally, these receptors are Type I transmembrane receptors with one or more cysteine rich repeat sequences in their extracellular domain. The TNF receptor superfamily may be further subdivided into (1) death receptors; (2) decoy receptors; and (3) signaling receptors that lack death domains. The "death receptors" contain in their cytoplasmic or intracellular region a "death domain", *i.e.*, a region or sequence which acts to transduce signals in the cell which can result in apoptosis or in induction of certain genes. The "decoy receptors" lack a functional death domain and are incapable of transducing signals which result in apoptosis. Examples of cytokines in the TNF gene family include Tumor Necrosis Factor- alpha (TNF-alpha), Tumor Necrosis Factor-beta (TNF-beta or lymphotoxin), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as TRAIL), Apo-3 ligand (also referred to as TWEAK), osteoprotegerin (OPG), APRIL, RANK ligand (also referred to as TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK). Examples of receptors in the TNF receptor superfamily include: type 1 Tumor Necrosis Factor Receptor (TNFR1), type 2 Tumor Necrosis Factor Receptor (TNFR2), p75 Nerve Growth Factor receptor (NGFR), the B cell surface antigen CD40, the T cell antigen OX-40, Apo-1 receptor (also called Fas or CD95), Apo-3 receptor (also called DR3, swl-1, TRAMP and LARD), the receptor called "Transmembrane

Activator and CAML-Interactor" or "TACI", BCMA protein, DR4, DR5 (alternatively referred to as Apo-2; TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER), DR6, DcR1 (also referred to as TRID, LIT or TRAIL-R3), DcR2 (also called TRAIL-R4 or TRUNDD), OPG, DcR3 (also called TR6 or M68), CAR1, HVEM (also called ATAR or TR2), GITR, ZTNFR-5, NTR-1, TNFL1, CD30, Lymphotoxin beta receptor (LTBr), 4-1BB receptor and TR9 (EP988, 371A1).

The terms "Apo-2 ligand", "Apo-2L", "Apo2L", Apo-2 ligand/TRAIL" and "TRAIL" are used herein interchangeably to refer to a polypeptide sequence which includes amino acid residues 114-281, inclusive, 95-281, inclusive, residues 92-281, inclusive, residues 91-281, inclusive, residues 41-281, inclusive, residues 39-281, inclusive, residues 15-281, inclusive, or residues 1-281, inclusive, of the amino acid sequence shown in Fig. 24 (SEQ ID No. 46), as well as biologically active fragments, deletional, insertional, and/or substitutional variants of the above sequences. In one embodiment, the polypeptide sequence comprises residues 114-281 of Fig. 24 (SEQ ID No. 46). Optionally, the polypeptide sequence comprises residues 92-281 or residues 91-281 of Fig. 24 (SEQ ID No. 46). The Apo-2L polypeptides may be encoded by the native nucleotide sequence shown in Fig. 24 (SEQ ID No. 45). Optionally, the codon which encodes residue Pro119 (Fig. 24; SEQ ID No. 45) may be "CCT" or "CCG". Optionally, the fragments or variants are biologically active and have at least about 80% amino acid sequence identity, or at least about 90% sequence identity, or at least 95%, 96%, 97%, 98%, or 99% sequence identity with any one of the above sequences. The definition encompasses substitutional variants of Apo-2 ligand in which at least one of its native amino acids are substituted by another amino acid such as an alanine residue. The definition also encompasses a native sequence Apo-2 ligand isolated from an Apo-2 ligand source or prepared by recombinant and/or synthetic methods. The Apo-2 ligand of the invention includes the polypeptides referred to as Apo-2 ligand or TRAIL disclosed in WO97/01633 published January 16, 1997, WO97/25428 published July 17, 1997, WO99/36535 published July 22, 1999, WO 01/00832 published January 4, 2001, WO02/09755 published February 7, 2002, WO 00/75191 published December 14, 2000, and U.S. Patent No. 6,030,945 issued February 29, 2000. The terms are used to refer generally to forms of the Apo-2 ligand which include monomer, dimer, trimer, hexamer or hight oligomer forms of the polypeptide. All numbering of amino acid residues referred to in the Apo-2L sequence use the numbering according to Fig. 24 (SEQ ID No. 46), unless specifically stated otherwise.

"Apo-2 ligand receptor" includes the receptors referred to in the art as "DR4" and "DR5." *Pan et al.* have described the TNF receptor family member referred to as "DR4" (Pan *et al.*, *Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO 99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002). Sheridan *et al.*, *Science*, 277:818-821 (1997) and Pan *et al.*, *Science*, 277:815-818 (1997) described another receptor for Apo2L/TRAIL (see also, WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998). This receptor is referred to as DR5 (the receptor has also been alternatively referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER; Screaton *et al.*, *Curr. Biol.*, 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924

published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003). As described above, other receptors for Apo-2L include DcR1, DcR2, and OPG. The term "Apo-2L receptor" when used herein encompasses native sequence receptor and receptor variants. These terms encompass Apo-2L receptor expressed in a variety of mammals, including humans. Apo-2L receptor may be endogenously expressed as occurs naturally in a variety of human tissue lineages, or may be expressed by recombinant or synthetic methods. A "native sequence Apo-2L receptor" comprises a polypeptide having the same amino acid sequence as an Apo-2L receptor derived from nature. Thus, a native sequence Apo-2L receptor can have the amino acid sequence of naturally-occurring Apo-2L receptor from any mammal, including humans. Such native sequence Apo-2L receptor can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence Apo-2L receptor" specifically encompasses naturally-occurring truncated or secreted forms of the receptor (*e.g.*, a soluble form containing, for instance, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants. Receptor variants may include fragments or deletion mutants of the native sequence Apo-2L receptor. Figs. 25A-C show the 411 amino acid sequence of human DR5 receptor, along with its nucleotide sequence (SEQ ID Nos. 47 and 48) as published in WO 98/51793 on November 19, 1998. A transcriptional splice variant of human DR5 receptor is known in the art. This splice variant encodes the 440 amino acid sequence of human DR5 receptor as shown in Figs. 26A-C, along with its nucleotide sequence (SEQ ID Nos. 49 and 50), and as published in WO 98/35986 on August 20, 1998.

"Death receptor antibody" is used herein to refer generally to antibody or antibodies directed to a receptor in the tumor necrosis factor receptor superfamily and containing a death domain capable of signalling apoptosis, and such antibodies include DR5 antibody and DR4 antibody.

"DR5 receptor antibody", "DR5 antibody", or "anti-DR5 antibody" is used in a broad sense to refer to antibodies that bind to at least one form of a DR5 receptor or extracellular domain thereof. Optionally the DR5 antibody is fused or linked to a heterologous sequence or molecule. Preferably the heterologous sequence allows or assists the antibody to form higher order or oligomeric complexes. Optionally, the DR5 antibody binds to DR5 receptor but does not bind or cross-react with any additional Apo-2L receptor (*e.g.* DR4, DcR1, or DcR2). Optionally the antibody is an agonist of DR5 signalling activity.

Optionally, the DR5 antibody of the invention binds to a DR5 receptor at a concentration range of about 0.1 nM to about 20 mM as measured in a BIAcore binding assay. Optionally, the DR5 antibodies of the invention exhibit an IC50 value of about 0.6 nM to about 18 mM as measured in a BIAcore binding assay.

Purely for the purposes herein, the term "Apomab" refers to an agonist antibody which binds to DR5 and comprises the variable heavy and variable light amino acid sequences of SEQ ID Nos. 55 and 56. Preferably Apomab comprises the heavy and light chains of SEQ ID Nos. 51 and 52, respectively.

II. Production of Antibodies

Techniques for producing antibodies which can be formulated according to the present invention follow.

(i) Antigen selection and preparation

Preferably, the antigen to which the antibody binds is a biologically important glycoprotein and administration of the antibody to a mammal suffering from a disease or disorder can result in a therapeutic benefit in that mammal. However, antibodies directed against nonpolypeptide antigens (such as tumor-associated glycolipid antigens; see US Patent 5,091,178) are also contemplated.

Where the antigen is a polypeptide, it may be a transmembrane molecule (*e.g.* receptor) or ligand such as a growth factor. Exemplary antigens include molecules such as renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor (TF), and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-b; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-b1, TGF-b2, TGF-b3, TGF-b4, or TGF-b5; a tumor necrosis factor (TNF) such as TNF-alpha or TNF-beta; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19, CD20, CD22 and CD40; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 and IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4 and VCAM; a tumor associated antigen such as HER2, HER3 or HER4 receptor; and fragments of any of the above-listed polypeptides.

Exemplary molecular targets for antibodies encompassed by the present invention include CD proteins such as CD3, CD4, CD8, CD19, CD20, CD22, CD34 and CD40; members of the ErbB receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; B cell surface antigens, such as CD20 or BR3; a member of the tumor necrosis receptor superfamily, including DR5; prostate stem cell antigen (PSCA); cell adhesion molecules such as LFA-1, Mac1, p150.95, VLA-4, ICAM-1, VCAM, alpha4/beta7 integrin, and alphav/beta3

integrin including either alpha or beta subunits thereof (*e.g.* anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF as well as receptors therefor; tissue factor (TF); a tumor necrosis factor (TNF) such as TNF-alpha or TNF-beta, alpha interferon (alpha-IFN); an interleukin, such as IL-8; IgE; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C etc.

Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (*e.g.* the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (*e.g.* cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule. Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

For production of HER2 antibodies, the HER2 antigen to be used for production thereof may be, *e.g.*, a soluble form of the extracellular domain of HER2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing HER2 at their cell surface (*e.g.* NIH-3T3 cells transformed to overexpress HER2; or a carcinoma cell line such as SK-BR-3 cells, see Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991)) can be used to generate antibodies.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

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

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11

mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); 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. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

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 preferred 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. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

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.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) 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.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). 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 (U.S. Patent No. 4,816,567;

and Morrison, *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), 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.

(iii) Humanized antibodies

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

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

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

WO01/00245 describes production of exemplary humanized HER2 antibodies which bind HER2 and block ligand activation of a HER receptor. The humanized antibody of particular interest herein blocks EGF, TGF-α and/or HRG mediated activation of MAPK essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof) and/or binds HER2 essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof). The humanized antibody herein 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.

An exemplary humanized antibody of interest herein comprises variable heavy domain complementarity determining residues GFTFTDYTMX, where X is preferably D or S (SEQ ID No. 7); DVNPNSGGSIYNQRFKG (SEQ ID No. 8); and/or NLGPSFYFDY (SEQ ID No. 9), optionally comprising amino acid modifications of those CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable heavy CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable heavy domain amino acid sequence in SEQ ID No. 4.

The humanized antibody may comprise variable light domain complementarity determining residues KASQDVSIGVA (SEQ ID No. 10); SASYXXX, where the X at position 5 is preferably R or L, wherein the X at position 6 is preferably Y or E, and the X at position 7 is preferably T or S (SEQ ID No. 11); and/or QQYYIYPYT (SEQ ID No. 12), *e.g.* in addition to those variable heavy domain CDR residues in the preceding paragraph. Such humanized antibodies optionally comprise amino acid modifications of the above CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable light CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable light domain amino acid sequence in SEQ ID No. 3.

The present application also contemplates affinity matured antibodies which bind HER2 and block ligand activation of a HER receptor. The parent antibody may be a human antibody or a humanized antibody, *e.g.*, one comprising the variable light and/or heavy sequences of SEQ ID Nos. 3 and 4, respectively (*i.e.* variant 574). The affinity matured antibody preferably binds to HER2 receptor with an affinity superior to that of murine 2C4 or variant 574 (*e.g.* from about two or about four fold, to about 100 fold or about 1000 fold improved affinity, *e.g.* as assessed using a HER2-extracellular domain (ECD) ELISA). Exemplary variable heavy CDR residues for substitution include H28, H30, H34, H35, H64, H96, H99, or combinations of two or more (*e.g.* two, three, four, five, six, or seven of these residues). Examples of variable light CDR residues for alteration include L28, L50, L53, L56, L91, L92, L93, L94, L96, L97 or combinations of two or more (*e.g.* two to three, four, five or up to about ten of these residues).

Various forms of the humanized antibody or affinity matured antibody are contemplated. For example, the humanized antibody or affinity matured antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody or affinity matured antibody may be an full length antibody, such as an full length IgG1 antibody.

(iv) Human antibodies

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. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); Bruggermann *et al., Year in Immuno.*, 7:33 (1993); and U.S. Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty *et al., Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al., Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. 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 following the techniques described by Marks *et al., J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al., EMBO J.* 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

Human HER2 antibodies are described in U.S. Patent No. 5,772,997 issued June 30, 1998 and WO 97/00271 published January 3, 1997.

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of full length antibodies (see, e.g., Morimoto *et al.* , *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al., Science*, 229:81 (1985)).

However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. 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; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the HER2 protein. Other such antibodies may combine a HER2 binding site with binding site(s) for EGFR, HER3 and/or HER4. Alternatively, a HER2 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 HER2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express HER2. These antibodies possess a HER2-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies).

WO 96/16673 describes a bispecific HER2/FcγRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific HER2/FcγRI antibody IDM1 (Osidem). A bispecific HER2/Fcα antibody is shown in WO98/02463. U.S. Patent No. 5,821,337 teaches a bispecific HER2/CD3 antibody. MDX-210 is a bispecific HER2-FcγRIII Ab.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

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

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

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

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein full length antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the

production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

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

(vii) Other amino acid sequence modifications

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the Antibody are prepared by introducing appropriate nucleotide changes into the Antibody nucleic acid, 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 called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed Antibody variants are screened for the desired activity.

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

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

Table 1

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

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to

similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

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

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

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

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

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

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

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

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

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

(6) aromatic: Trp, Tyr, Phe.

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

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).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e. g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and its antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

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

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 A1, Presta, L. See also US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO03/011878, Jean-Mairet *et al.* and US Patent No. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO97/30087, Patel *et al.* See, also, WO98/58964 (Raju, S.) and WO99/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. Antibody compositions comprising main species antibody with such carbohydrate structures attached to the Fc region are contemplated herein.

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.

(viii) Screening for antibodies with the desired properties

Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

To identify an antibody which blocks ligand activation of a HER receptor, the ability of the antibody to block HER ligand binding to cells expressing the HER receptor (*e.g.* in conjugation with another HER receptor with which the HER receptor of interest forms a HER hetero-oligomer) may be determined. For example, cells naturally expressing, or transfected to express, HER receptors of the HER hetero-oligomer may be incubated with the antibody and then exposed to labeled HER ligand. The ability of the HER2 antibody to block ligand binding to the HER receptor in the HER hetero-oligomer may then be evaluated.

For example, inhibition of HRG binding to MCF7 breast tumor cell lines by HER2 antibodies may be performed using monolayer MCF7 cultures on ice in a 24-well-plate format essentially as described in WO01/00245. HER2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRGβ1$_{177-224}$ (25 pm) 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 antibody of interest. In one embodiment, the antibody which blocks ligand activation of an HER receptor will have an IC_{50} for inhibiting HRG binding to MCF7 cells in this assay of about 50nM or less, more preferably 10nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG binding to MCF7 cells in this assay may, for example, be about 100nM or less, more preferably 50nM or less.

Alternatively, or additionally, the ability of the HER2 antibody to block HER ligand-stimulated tyrosine phosphorylation of a HER receptor present in a HER hetero-oligomer may be assessed. For example, cells endogenously expressing the HER receptors or transfected to expressed them may be incubated with the antibody and then assayed for HER ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S. Patent No. 5,766,863 is also available for determining HER receptor activation and blocking of that activity by an antibody.

In one embodiment, one may screen for an antibody which inhibits HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described in WO01/00245. For example, the MCF7 cells may be plated in 24-well plates and monoclonal antibodies to HER2 may be added to each well and incubated for 30 minutes at room temperature; then rHRG$\beta 1_{177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for 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. The antibody selected will preferably significantly inhibit HRG stimulation of p180 tyrosine phosphorylation to about 0-35% of 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 antibody of interest may be calculated. In one embodiment, the antibody which blocks ligand activation of a HER receptor will have an IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay of about 50nM or less, more preferably 10nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay may, for example, be about 100nM or less, more preferably 50nM or less.

One may also assess the growth inhibitory effects of the antibody on MDA-MB-175 cells, *e.g.*, essentially as described in Schaefer *et al. Oncogene* 15:1385-1394 (1997). According to this assay, MDA-MB-175 cells may treated with a HER2 monoclonal antibody (10µg/mL) for 4 days and stained with crystal violet. Incubation with a HER2 antibody may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4. In a further embodiment, exogenous HRG will not significantly reverse this inhibition. Preferably, the antibody will be able to inhibit cell proliferation of MDA-MB-175 cells to a greater extent than monoclonal antibody 4D5 (and optionally to a greater extent than monoclonal antibody 7F3), both in the presence and absence of exogenous HRG.

In one embodiment, the HER2 antibody of interest may block heregulin dependent association of HER2 with HER3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment such as that described in WO01/00245 substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

To identify growth inhibitory HER2 antibodies, one may screen for antibodies which inhibit the growth of cancer cells which overexpress HER2. In one embodiment, the growth inhibitory antibody of choice is able to inhibit growth of SK-BR-3 cells in cell culture by about 20-100% and preferably by about 50-100% at an antibody concentration of about 0.5 to 30 μg/ml. To identify such antibodies, the SK-BR-3 assay described in U.S. Patent No. 5,677,171 can be performed. According to this assay, SK-BR-3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The SK-BR-3 cells are plated at 20,000 cells in a 35mm cell culture dish (2mls/35mm dish). 0.5 to 30 μg/ml of the HER2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SK-BR-3 cells by about 20-100% or about 50-100% may be selected as growth inhibitory antibodies. See US Pat No. 5,677,171 for assays for screening for growth inhibitory antibodies, such as 4D5 and 3E8.

In order to select for HER2 antibodies which induce apoptosis, an annexin binding assay using BT474 cells is available. The BT474 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 10μg/ml of the monoclonal antibody. 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) (1μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing antibodies. In addition to the annexin binding assay, a DNA staining assay using BT474 cells is available. In order to perform this assay, BT474 cells which have been treated with the antibody of interest as described in the preceding two paragraphs are incubated with 9μg/ml HOECHST 33342™ for 2 hr at 37°C, then analyzed on an EPICS ELITE™ flow cytometer (Coulter Corporation) using MODFIT LT™ software (Verity Software House). Antibodies which induce a change in the percentage of apoptotic cells which is 2 fold or greater (and preferably 3 fold or greater) than untreated cells (up to 100% apoptotic cells) may be selected as pro-apoptotic antibodies using this assay. See WO98/17797 for assays for screening for HER2 antibodies which induce apoptosis, such as 7C2 and 7F3.

To screen for antibodies which bind to an epitope on HER2 bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed to assess whether the antibody cross-blocks binding of an antibody, such as 2C4 or Pertuzumab, to HER2. Alternatively, or additionally, epitope mapping can be performed by methods known in the art and/or one can study the antibody-HER2 structure (Franklin *et al. Cancer Cell* 5:317-328 (2004)) to see what domain(s) of HER2 is/are bound by the antibody.

(ix) Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. a small molecule toxin or an enzymatically active toxin of

bacterial, fungal, plant or animal origin, including fragments and/or variants thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (U.S. Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein.

In one preferred embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (*e.g.* about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al. Cancer Research* 52: 127-131 (1992)) to generate a maytansinoid-antibody immunoconjugate.

Another immunoconjugate of interest comprises a HER2 antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)). See, also, US Patent Nos. 5,714,586; 5,712,374; 5,264,586; and 5,773,001 expressly incorporated herein by reference.

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

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

A variety of radioactive isotopes are available for the production of radioconjugated HER2 antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used.

Alternatively, a fusion protein comprising the HER2 antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

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

(x) Other antibody modifications

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may 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, Oslo, A., Ed., (1980).

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cyotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

WO00/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof. Preferably, the antibody with improved ADCC comprises substitutions at positions 298, 333, and/or 334 of the Fc region. Preferably the altered Fc region is a human IgG1 Fc region comprising or consisting of substitutions at one, two or three of these positions.

Antibodies with altered C1q binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof.

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 Patent 5,739,277, 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. Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO00/42072 (Presta, L.).

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 A1, Miller *et al.*).

The HER2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.* 81(19)1484 (1989).

(ix) Exemplary Antibodies

Exemplary antibodies which can be formulated according to the present invention include, but are not limited to the following:

anti-ErbB antibodies, including anti-HER2 antibodies, such as those described in more detail herein;

antibodies that bind to a B-cell surface marker, such as CD19, CD20 (for example Rituximab (RITUXAN®) and humanized 2H7), CD22, CD40 or BR3;

antibodies that bind to IgE, including Omalizumab (XOLAIR®) commercially available from Genentech, E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies. See, also, Presta *et al., J. Immunol.* 151:2623-2632 (1993); International Publication No. WO 95/19181; US Patent No. 5,714,338, issued February 3, 1998; US Patent No. 5,091,313, issued February 25, 1992; WO 93/04173 published March 4, 1993; WO 99/01556 published January 14, 1999; and US Patent No. 5,714,338;

antibodies that bind to vascular endothelial growth factor (VEGF) or a receptor thereof, including Bevacizumab (AVASTIN™), commercially available from Genentech, and Ranibizumab (LUCENTIS™);

anti-IL-8 antibodies (St John *et al., Chest*, 103:932 (1993), and International Publication No. WO 95/23865);

anti-PSCA antibodies (WO01/40309);

anti-CD40 antibodies, including S2C6 and humanized variants thereof (WO00/75348);

anti-CD11a antibodies, including efalizumab (RAPTIVA®) (US Patent No. 5,622,700, WO 98/23761, Steppe *et al., Transplant Intl.* 4:3-7 (1991), and Hourmant *et al., Transplantation* 58:377-380 (1994)); anti-CD18 antibodies (US Patent No. 5,622,700, issued April 22, 1997, or as in WO 97/26912, published July 31, 1997);

anti-Apo-2 receptor antibody (WO 98/51793 published November 19, 1998);

anti-TNF-alpha antibodies including cA2 (REMICADE®), CDP571 and MAK-195 (See, US Patent No. 5,672,347 issued September 30, 1997, Lorenz *et al. J. Immunol.* 156(4):1646-1653 (1996), and Dhainaut *et al. Crit. Care Med.* 23(9):1461-1469 (1995));

anti-Tissue Factor (TF) (European Patent No. 0 420 937 B1 granted November 9, 1994);

anti-human $\alpha 4\beta_7$ integrin (WO 98/06248 published February 19, 1998);
anti-EGFR antibodies, including chimerized or humanized 225 antibody as in WO 96/40210 published December 19, 1996;
anti-CD3 antibodies, such as OKT3 (US Patent No. 4,515,893 issued May 7, 1985);
anti-CD25 or anti-tac antibodies such as CHI-621 (SIMULECT®) and (ZENAPAX®) (See US Patent No. 5,693,762 issued December 2, 1997);
anti-CD4 antibodies such as the cM-7412 antibody (Choy *et al. Arthritis Rheum* 39(1):52-56 (1996));
anti-CD52 antibodies such as CAMPATH-1H (Riechmann *et al. Nature* 332:323-337 (1988));
anti-Fc receptor antibodies such as the M22 antibody directed against FcγRI as in Graziano *et al. J. Immunol.* 155(10):4996-5002 (1995);
anti-carcinoembryonic antigen (CEA) antibodies such as hMN-14 (Sharkey *et al. Cancer Res.* 55(23Suppl): 5935s-5945s (1995);
antibodies directed against breast epithelial cells including huBrE-3, hu-Mc 3 and CHL6 (Ceriani *et al. Cancer Res.* 55(23): 5852s-5856s (1995); and Richman *et al. Cancer Res.* 55(23 Supp): 5916s-5920s (1995));
antibodies that bind to colon carcinoma cells such as C242 (Litton *et al. Eur J. Immunol.* 26(1):1-9 (1996));
anti-CD38 antibodies, *e.g.* AT 13/5 (Ellis *et al. J. Immunol.* 155(2):925-937 (1995));
anti-CD33 antibodies such as Hu M195 (Jurcic *et al. Cancer Res* 55(23 Suppl):5908s-5910s (1995) and CMA-676 or CDP771;
anti-CD22 antibodies such as LL2 or LymphoCide (Juweid *et al. Cancer Res* 55(23 Suppl):5899s-5907s (1995);
anti-EpCAM antibodies such as 17-1A (PANOREX®);
anti-GpIIb/IIIa antibodies such as abciximab or c7E3 Fab (REOPRO®);
anti-RSV antibodies such as MEDI-493 (SYNAGIS®);
anti-CMV antibodies such as PROTOVIR®;
anti-HIV antibodies such as PRO542;
anti-hepatitis antibodies such as the anti-Hep B antibody OSTAVIR®;
anti-CA 125 antibody OvaRex;
anti-idiotypic GD3 epitope antibody BEC2;
anti-$\alpha v\beta 3$ antibody VITAXIN®;
anti-human renal cell carcinoma antibody such as ch-G250; ING-1;
anti-human 17-1A antibody (3622W94);
anti-human colorectal tumor antibody (A33);
anti-human melanoma antibody R24 directed against GD3 ganglioside;
anti-human squamous-cell carcinoma (SF-25); and
anti-human leukocyte antigen (HLA) antibodies such as Smart ID10 and the anti-HLA DR antibody Oncolym (Lym-1).

(xi) Antibody variant compositions

The present invention, in at least one aspect, concerns formulations comprising a composition which comprises a mixture of a main species antibody and one or more variants thereof. Where the main species antibody binds HER2, preferably the HER2 antibody (either or both of the main species HER2 antibody and antibody variant thereof) is one which binds to Domain II of HER2, inhibits HER dimerization more effectively

than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of the main species antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

In one embodiment, the formulated HER2 antibody composition comprises a mixture of the main species HER2 antibody and an amino acid sequence variant thereof comprising an amino-terminal leader extension. Preferably, the amino-terminal leader extension is on a light chain of the antibody variant (*e.g.* on one or two light chains of the antibody variant). The main species HER2 antibody or the antibody variant may be an full length antibody or antibody fragment (*e.g.* Fab or F(ab')2 fragments), but preferably both are full length antibodies. The antibody variant herein may comprise an amino-terminal leader extension on any one or more of the heavy or light chains thereof. Preferably, the amino-terminal leader extension is on one or two light chains of the antibody. The amino-terminal leader extension preferably comprises or consists of VHS-. Presence of the amino-terminal leader extension in the composition can be detected by various analytical techniques including, but not limited to, N-terminal sequence analysis, assay for charge heterogeneity (for instance, cation exchange chromatography or capillary zone electrophoresis), mass spectrometry, etc. The amount of the antibody variant in the composition generally ranges from an amount that constitutes the detection limit of any assay (preferably N-terminal sequence analysis) used to detect the variant to an amount less than the amount of the main species antibody. Generally, about 20% or less (*e.g.* from about 1% to about 15%, for instance from 5% to about 15%) of the antibody molecules in the composition comprise an amino-terminal leader extension. Such percentage amounts are preferably determined using quantitative N-terminal sequence analysis or cation exchange analysis (preferably using a high-resolution, weak cation-exchange column, such as a PROPAC WCX-10™ cation exchange column). Aside from the amino-terminal leader extension variant, further amino acid sequence alterations of the main species antibody and/or variant are contemplated, including but not limited to an antibody comprising a C-terminal lysine residue on one or both heavy chains thereof, a deamidated antibody variant, etc.

Moreover, the main species antibody or variant may further comprise glycosylation variations, non-limiting examples of which include HER2 antibody comprising a G1 or G2 oligosaccharide structure attached to the Fc region thereof, HER2 antibody comprising a carbohydrate moiety attached to a light chain thereof (*e.g.* one or two carbohydrate moieties attached to one or two light chains of the antibody), HER2 antibody comprising a non-glycosylated heavy chain.

III. Preparation of the Formulation

The present invention provides, in a first aspect, a stable pharmaceutical formulation comprising a monoclonal antibody, preferably a full length human or humanized IgG1 antibody, in histidine-acetate buffer, pH 5.5 to 6.5, preferably pH 5.8 to 6.2. However, the antibody in the formulation may be an antibody fragment comprising an antigen-binding region, such as a Fab or F(ab')2 fragment.

In another embodiment, the invention concerns a pharmaceutical formulation comprising, or consisting essentially of, a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; histidine-acetate buffer, pH 5.5 to 6.5; saccharide selected from the group

consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and polysorbate 20 in an amount from about 0.01% to about 0.1%.

In yet a further embodiment, the invention provides a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant. For example, the formulation may comprise Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5

In another aspect, the invention provides a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant. Such a formulation may, for example, comprise, Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The formulation is especially useful for antibodies that are susceptible to deamidation and/or aggregation and/or fragmentation, in that the buffer retards deamidation and/or aggregation and/or fragmentation of the antibody formulated therein. In addition, unlike other histidine buffers prepared using HCl, the histidine-acetate buffer lacks the chloride ion which was found to be beneficial herein in that this buffer when combined with saccharide had the same protective effect on antibody as polysorbate 20, and was stable and compatible with storage in stainless steel tanks. Thus, in addition to the formulation *per se* comprising the antibody susceptible to deamidation, aggregation and/or fragmentation, the invention provides a method for reducing deamidation, aggregation and/or fragmentation of a therapeutic monoclonal antibody (for example, relative to a composition at a different pH or in a different buffer), comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5. In this embodiment, one may determine or measure deamidation, aggregation and/or fragmentation before and after the antibody is formulated, with the formulated antibody demonstrating acceptable deamidation, aggregation and/or fragmentation in the formulation and upon storage thereof.

The antibody in the formulation may bind an antigen including but not limited to: HER2, CD20, IgE, DR5, BR3 and VEGF.

Where the formulated antibody binds HER2, it preferably is one which binds to Domain II of HER2, inhibits HER dimerization more effectively than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of a formulated HER2 antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 15 and 16 (Pertuzumab).

Examples of CD20 antibodies which can be formulated herein include: "C2B8" which is now called "Rituximab" ("RITUXAN®") commercially available from Genentech (see also US Patent No. 5,736,137, expressly incorporated herein by reference); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" ZEVALIN® commercially available from Biogen-Idec (see also US Patent No. 5,736,137, expressly incorporated herein by reference); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "131I-B1" antibody (iodine I131 tositumomab, BEXXAR™) (US Patent No. 5,595,721, expressly incorporated herein by reference); murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework patched" or humanized 1F5 (WO03/002607, Leung, S.); ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (Clark *et al. PNAS* 82: 1766-1770 (1985); US Patent No. 5,500,362, expressly incorporated herein by reference); humanized

2H7; huMax-CD20 (WO 04/035607, Genmab, Denmark); AME-133 (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: Leukocyte Typing III (McMichael, Ed., p. 440, Oxford University Press (1987)).

In the preferred embodiment of a formulated CD20 antibody, the CD20 antibody is a humanized 2H7 antibody. Prefered humanized 2H7 antibodies herein are 2H7v16 and 2H7v511. The humanized 2H7v16 may be an intact antibody or antibody fragment comprising the variable light and variable heavy sequences in Figs. 18A-B (SEQ ID Nos. 26 and 29). Where the humanized 2H7v16 antibody is a full length antibody, preferably it comprises the light and heavy chain amino acid sequences with SEQ ID Nos. 63 and 65.

Where the antibody binds VEGF, it preferably comprises the variable domain sequences as depicted in Fig. 19. The most preferred anti-VEGF antibody is full length humanized IgG1antibody, Bevacizumab (AVASTIN™), commercially available from Genentech.

Where the formulated antibody binds IgE, it is preferably selected from the group consisting of: E25, Omalizumab (XOLAIR®) commercially available from Genentech (see also Figs. 17A-B), E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies.

Where the antibody binds to a receptor in the tumor necrosis factor (TNF) superfamily or to a death receptor, it preferably binds to DR5, and preferably is an agonist antibody. Publications in this area include Sheridan *et al.*, *Science*, 277:818-821 (1997), Pan *et al.*, *Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton *et al.*, *Curr. Biol.*, 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/012555540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; US 6,743,625 issued June 1, 2004. The most preferred DR5 antibody is Apomab.

Each of the formulations noted above comprises a buffer, preferably a histidine buffer, and most preferably a histidine-acetate buffer with a pH of 5.5 to 6.5, preferably 5.8 to 6.2, for example approximately 6.0. The concentration of the buffer is dictated, at least in part, by the desired pH. Exemplary concentrations for the buffer are in the range from about 1mM to about 200mM, preferably from about 10mM to about 40mM, most preferably about 20mM.

The antibody concentration in the formulation is preferably in the range from about 10mg/mL to about 250mg/mL. The antibody concentration may be determined based on the intended use and mode of administration of the formulation. For example, where the formulation is for IV administration (*e.g.*, a HER2

antibody), the antibody concentration in the formulation is preferably from about 20mg/mL to about 40mg/mL. In the exemplified Pertuzumab formulation intended for intravenous (IV) administration, the antibody concentration was from about 20mg/mL to about 40mg/mL, most preferably about 30mg/mL.

Where the antibody is for SQ or IM administration (*e.g.* for an anti-IgE antibody) higher concentrations of the antibody may be desired. Such substantially high antibody concentrations may be from about 50mg/mL to about 250mg/mL, or from about 80mg/mL to about 250mg/mL, or from about 100mg/mL to about 200mg/mL.

Where the formulation comprises a DR5 antibody, such as Apomab, exemplary antibody concentrations are from about 10mg/mL to about 30mg/mL, for example about 20mg/mL DR5 antibody; such formulation being useful for intravenous administration.

The formulation for administration is preferably an aqueous formulation (not lyophilized) and has not been subjected to prior lyophilization. While the formulation may be lyophilized, preferably it is not. However, freezing of the aqueous formulation, without simultaneous drying that occurs during freeze-drying, is specifically contemplated herein, facilitating longer term storage thereof, for instance in a stainless steel tank.

The formulation preferably further comprises a saccharide, most preferably a disaccharide, such as trehalose or sucrose. The saccharide is generally included in an amount which reduces soluble aggregate formation, such as that which occurs upon freeze/thaw. Exemplary saccharide concentrations are in the range from about 10mM to about 1M, for example from about 60mM to about 250mM, and most preferably about 120mM for a HER2 antibody formulation, and about 240mM for a DR5 antibody formulation.

While it was found herein that a formulation comprising histidine-acetate buffer and saccharide was stable, the formulation optionally further comprises surfactant, such as polysorbate, most preferably polysorbate 20. The surfactant is generally included in an amount which reduces insoluble aggregate formation (such as that which occurs upon shaking or shipping). The surfactant concentration is preferably from about 0.0001% to about 1.0%, most preferably from about 0.01% to about 0.1%, for example about 0.02%.

Optionally, the formulation does not contain a tonicifying amount of a salt such as sodium chloride.

The formulation is generally sterile, and this can be achieved according to the procedures known to the skilled person for generating sterile pharmaceutical formulations suitable for administration to human subjects, including filtration through sterile filtration membranes, prior to, or following, preparation of the formulation.

Moreover, the formulation is desirably one which has been demonstrated to be stable upon storage. Various stability assays are available to the skilled practitioner for confirming the stability of the formulation. For example, the formulation may be one which is found to be stable upon storage: at about 40°C for at least 4 weeks; at about 5°C or about 15°C for at least 3 months or at least 1 year; and/or about -20°C for at least 3 months. Stability can be tested by evaluating physical stability, chemical stability, and/or biological activity of the antibody in the formulation around the time of formulation as well as following storage at the noted temperatures. Physical and/or stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluting biological activity or antigen binding function of the antibody; etc. Instability may result in aggregation, deamidation (*e.g.* Asn deamidation), oxidation (*e.g.* Met oxidation),

isomerization (*e.g.* Asp isomeriation), clipping/hydrolysis/fragmentation (*e.g.* hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc. Biological activity or antigen binding function can be evaluated using various techniques available to the skilled practitioner.

As noted above, freezing of the formulation is specifically contemplated herein. Hence, the formulation can be tested for stability upon freezing and thawing.

According, the invention also provides a method of making a pharmaceutical formulation comprising preparing the formulation as described herein, and evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

In the preferred embodiment, the formulation is provided inside a vial with a stopper pierceable by a syringe, preferably in aqueous form. The vial is desirably stored at about 2-8°C until it is administered to a subject in need thereof. The vial may for example be a 20cc vial (for example for a 420mg dose) or 50cc vial (for example for a 1050mg dose). For a DR5 antibody, such as Apomab, the formulation may be provided in a 5cc glass vial (*e.g.* 5.5ml fill).

In another embodiment, the formulation is provided inside a stainless steel tank. The formulation in the stainless steel tank is optionally frozen and not freeze-dried.

One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (*e.g.* Zn-protein complexes); biodegradable polymers such as polyesters; preservatives; and/or salt-forming counterions such as sodium.

IV. Treatment with the Antibody Formulation

In one embodiment, the invention provides a method of treating a disease or disorder in a subject comprising administering the formulation described herein to a subject in an amount effective to treat the disease or disorder.

Where the antibody in the formulation binds to HER2, it is preferably used to treat cancer. The cancer will generally comprise HER2-expressing cells, such that the HER2 antibody herein is able to bind to the cancer cells. Thus, the invention in this embodiment concerns a method for treating HER2-expressing cancer in a subject, comprising administering the HER2 antibody pharmaceutical formulation to the subject in an amount effective to treat the cancer. Various cancers that can be treated with the composition are listed in the definitions section above.

It is also contemplated that the HER2 antibody formulation may be used to treat various non-malignant diseases or disorders, such a include autoimmune disease (*e.g.* psoriasis); endometriosis; scleroderma; restenosis; polyps such as colon polyps, nasal polyps or gastrointestinal polyps; fibroadenoma; respiratory disease (see definition above); cholecystitis; neurofibromatosis; polycystic kidney disease; inflammatory diseases; skin disorders including psoriasis and dermatitis; vascular disease (see definition above); conditions involving abnormal proliferation of vascular epithelial cells; gastrointestinal ulcers; Menetrier's disease, secreting

adenomas or protein loss syndrome; renal disorders; angiogenic disorders; ocular disease such as age related macular degeneration, presumed ocular histoplasmosis syndrome, retinal neovascularization from proliferative diabetic retinopathy, retinal vascularization, diabetic retinopathy, or age related macular degeneration; bone associated pathologies such as osteoarthritis, rickets and osteoporosis; damage following a cerebral ischemic event; fibrotic or edemia diseases such as hepatic cirrhosis, lung fibrosis, carcoidosis, throiditis, hyperviscosity syndrome systemic, Osler Weber-Rendu disease, chronic occlusive pulmonary disease, or edema following burns, trauma, radiation, stroke, hypoxia or ischemia; hypersensitivity reaction of the skin; diabetic retinopathy and diabetic nephropathy; Guillain-Barre syndrome; graft versus host disease or transplant rejection; Paget's disease; bone or joint inflammation; photoaging (*e.g.* caused by UV radiation of human skin); benign prostatic hypertrophy; certain microbial infections including microbial pathogens selected from adenovirus, hantaviruses, Borrelia burgdorferi, Yersinia spp. and Bordetella pertussis; thrombus caused by platelet aggregation; reproductive conditions such as endometriosis, ovarian hyperstimulation syndrome, preeclampsia, dysfunctional uterine bleeding, or menometrorrhagia; synovitis; atheroma; acute and chronic nephropathies (including proliferative glomerulonephritis and diabetes-induced renal disease); eczema; hypertrophic scar formation; endotoxic shock and fungal infection; familial adenomatosis polyposis; neurodedenerative diseases (*e.g.* Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration); myelodysplastic syndromes; aplastic anemia; ischemic injury; fibrosis of the lung, kidney or liver; T-cell mediated hypersensitivity disease; infantile hypertrophic pyloric stenosis; urinary obstructive syndrome; psoriatic arthritis; and Hasimoto's thyroiditis. Preferred non-malignant indications for therapy herein include psoriasis, endometriosis, scleroderma, vascular disease (*e.g.* restenosis, artherosclerosis, coronary artery disease, or hypertension), colon polyps, fibroadenoma or respiratory disease (*e.g.* asthma, chronic bronchitis, bronchiectasis or cystic fibrosis).

Where the antibody in the formulation binds to a B-cell surface marker such as CD20 or BAFF3, the formulation may be used to treat a B-cell malignancy, such as NHL or CLL, an autoimmune disease, graft rejection, or to block an immune response to a foreign antigen, such as an antibody, a toxin, a gene therapy viral vector, a graft, an infectious agent, or an alloantigen (see WO 01/03734, Grillo-Lopez *et al.*).

Where the antibody in the formulation is an IgE antibody, it may be used to treat an IgE-mediated disorder (USSN 2004/0197324 A1, Liu and Shire), such as allergic asthma, allergic rhinitis, atopic dermatitis, allergic gastroenteropathy, hypersensitivity, eczema, urticaria, allergic bronchopulmonary aspergillosis, parasitic disease, hyper-IgE syndrome, ataxia-telangiectasia, Wiskott-Aldrich syndrome, thymic alymphoplasia, IgE myeloma, and graft-versus-host reaction.

Antibodies that bind to a receptor in the TNF superfamily (for instance which bind to DR5), or which bind to VEGF (or a receptor thereof), may be used to treat cancer, various forms of which are described in the definitions section above. Preferably, the cancer treated with a DR5 antibody formulation is a solid tumor or NHL.

Where the indication is cancer, the patient may be treated with a combination of the antibody formulation, and a chemotherapeutic agent. The combined administration includes coadministration or concurrent administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Thus, the chemotherapeutic agent may be administered prior to,

or following, administration of the composition. In this embodiment, the timing between at least one administration of the chemotherapeutic agent and at least one administration of the composition is preferably approximately 1 month or less, and most preferably approximately 2 weeks or less. Alternatively, the chemotherapeutic agent and the composition are administered concurrently to the patient, in a single formulation or separate formulations.

Treatment with the formulation will result in an improvement in the signs or symptoms of cancer or disease. For instance, where the disease being treated is cancer, such therapy may result in an improvement in survival (overall survival and/or progression free survival) and/or may result in an objective clinical response (partial or complete). Moreover, treatment with the combination of the chemotherapeutic agent and the antibody formulation may result in a synergistic, or greater than additive, therapeutic benefit to the patient.

Preferably, the antibody in the formulation administered is a naked antibody. However, the antibody administered may be conjugated with a cytotoxic agent. Preferably, the immunoconjugate and/or antigen to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with nucleic acid in the cancer cell. Examples of such cytotoxic agents include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The formulation is administered to a human patient in accord with known methods, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous, intramuscular or subcutaneous administration of antibody composition is preferred, with intravenous administration being most preferred.

For subcutaneous delivery, the formulation may be administered via syringe; injection device (*e.g.* the INJECT-EASE™ and GENJECT™ device); injector pen (such as the GENPEN™); needleless device (*e.g.* MEDIJECTOR™ and BIOJECTOR™); or subcutaneous patch delivery system.

For the prevention or treatment of disease, the appropriate dosage of the antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody 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 antibody 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 50 mg/kg (*e.g.* 0.1-20mg/kg) of HER2 or DR5 antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. The dosage of the antibody will generally be in the range from about 0.05mg/kg to about 10mg/kg. If a chemotherapeutic agent is administered, it is usually administered at dosages known therefor, or optionally lowered due to combined action of the drugs or negative side effects attributable to administration of the chemotherapeutic agent. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

Other therapeutic regimens may be combined with the antibody including, but not limited to: a second (third, fourth, etc) chemotherapeutic agent(s) (*i.e.* "cocktails" of different chemotherapeutic agents); another

monoclonal antibody;a growth inhibitory agent; a cytotoxic agent; a chemotherapeutic agent; EGFR-targeted drug; tyrosine kinase inhibitor; anti-angiogenic agent; and/or cytokine; etc.

In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

V. Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the pharmaceutical formulation of the present invention and provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use as noted in the previous section.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations are incorporated herein by reference.

EXAMPLES

Stable Pertuzumab Liquid Formulations

These examples describe the development and stability testing of stable liquid formulations comprising Pertuzumab at protein concentrations in the range from about 10 mg/mL – 180 mg/mL. The selected formulations had low turbidity, and were physically and chemically stable. A chloride ion was removed from the formulation to reduce the risk of corrosion. The formulation was isotonic, and suitable for subcutaneous or intramuscular delivery. Insoluble aggregate formation upon agitation stress was prevented using histidine-acetate and sucrose formulation, without the need to include polysorbate 20.

Analytical Methods

Color, Appearance and Clarity (CAC)

The color, appearance, and clarity of the samples were determined by visual inspection of vials against a white and black background under white fluorescence light at room temperature.

UV Concentration Measurements

The liquid product aliquot was first diluted with formulation buffer so that the A_{max} near 278 nm is within 0.5-1.0 absorbance unit. The UV absorbance of the diluted samples was measured in a quartz cuvette with 1 cm path length on an HP 8453 spectrophotometer. Absorbance was measured at 278 nm and 320 nm. The absorbance from 320 nm is used to correct background light scattering due to larger aggregates, bubbles and

particles. The measurements were blanked against the formulation buffer. The protein concentration was determined using the absorptivity of 1.50 $(mg/mL)^{-1}cm^{-1}$.

pH measurements

The pH was measured at room temperature using a RADIOMETER COPENHAGEN PHM82™ pH meter. The probe used was a combined glass/reference electrode with radiometer connector (Sigma, Cat# E-5759). Standard solutions of pH 4.01 and pH 7.00 (EM Science) were used for calibration of the pH meter.

Ion-Exchange Chromatography (IEX)

Cation exchange chromatography was employed to measure changes in charge variants. This assay utilizes a DIONEX PROPAC WCX-10™ column on an HP 1100™ HPLC system. Samples were diluted to 1 mg/mL with the mobile phase A containing 20 mM MES at pH 6.0. 50 mL of diluted samples were then loaded on the column that was kept at ambient temperature. The peaks were eluted with a shallow NaCl gradient using mobile B containing 20 mM MES, 250 mM NaCl, pH 6.0. The eluent was monitored at 280 nm. The data were analyzed using HP CHEMSTATION™ software (Rev A08.03).

Capillary Zone Electrophophoresis (CZE)

The purity of Fab and $F(ab')_2$ fragments was determined by CZE. This assay was run on a BIORAD BIOFOCUS™ 3000™ capillary electrophoresis system with a BIOCAP XL™ capillary, 50 μm I.D., 44.6 cm total length and 40 cm to the detector.

Size Exclusion Chromatography (SEC)

Size exclusion chromatography was used to quantitate aggregates and fragments. This assay utilizes a TSK G3000 SWXL™, 7.8 x 300 mm column and runs on an HP 1100™ HPLC system. Samples were diluted to 10 mg/mL with the mobile phase and injection volume was 20 μL. The mobile phase was 100 mM K_2HPO_4 at pH 6.8 and the protein was eluted with an isocratic gradient at 0.5 mL/min for 45 minutes. The eluent absorbance was monitored at 280 nm. Integration was done using HP CHEMSTATION™ software (Rev A08.03).

Biological Activity

The biological activity of Pertuzumab was determined by measuring its ability to inhibit proliferation of the human breast cancer cell line MDA-MB-175-VII.

EXAMPLE 1

Pertuzumab Fab and $F(ab')_2$ antibody fragments were formulated at protein concentration of 1.0 mg/mL in the following buffer conditions:

10 mM citrate, 140 mM NaCl, pH 4.0;

10 mM succinate, 140 mM NaCl, pH 5.0;

10 mM succinate, 140 mM NaCl,, pH 6.0;

10 mM histidine, 140 mM NaCl, pH 7.0; and

10 mM glycylglycine, 140 mM NaCl, pH 8.0.

Each formulation was filtered then aliquoted into 3 cc WHEATON™ USP Type I glass vials sealed with TEFLON™ coated gray butyl stoppers. Samples were stored at 40 ± 2 °C. The stability analyses of drug product showed that the Fab and $F(ab')_2$ were most stable between pH 5.0 and 6.0.

Table 2. Effect of pH on degradation of Fab or $F(ab')_2$ stored at 40°C

Formulation pH	Fab		$F(ab')_2$	
	CZE % Main Peak	SEC % Main Peak	CZE % Main Peak	SEC % Main Peak
4.0	74.1	96.7	43.6	89.4
5.0	83.2	96.4	65.4	94.0
6.0	82.9	96.2	69.0	92.3
7.0	83.9	96.4	62.3	91.3
8.0	72.7	96.4	49.2	89.8

EXAMPLE 2

Pertuzumab was formulated into 20 mM histidine-acetate buffer with 120 mM sucrose and 0.02 % polysorbate 20. The pHs of formulations were adjusted with acetic acid to final pH between 5.0 and 7.0. The protein concentration was 30 mg/mL. Each formulation was filled into 3 cc USP Type I glass vials and stored at 40°C for stability analysis. The results showed that Pertuzumab was most stable around pH 6.0.

Table 3. Effect of pH on degradation of Pertuzumab stored at 40°C

Formulation pH	Temperature (°C)	Storage Time (wks)	SEC % Monomer	IEX % Main Peak
5.0	40	2	99.4	57.4
5.5	40	2	99.4	59.2
6.0	40	2	99.4	60.6
6.5	40	2	99.3	60.5
7.0	40	2	99.1	54.0
5.0	40	4	97.3	48.1
5.5	40	4	99.1	50.5
6.0	40	4	99.1	53.3
6.5	40	4	99.0	52.3
7.0	40	4	98.6	42.3

EXAMPLE 3

Pertuzumab formulations at protein concentration of 100 mg/mL were prepared in the following excipients:

(1) 10 mM histidine-HCl, 240 mM sucrose, 0.02% polysorbate 20, pH 6.0;

(2) 10 mM histidine-acetate, 240 mM sucrose, 0.02% polysorbate 20, pH 6.0;
(3) 10 mM histidine-phosphate, 240mM sucrose, 0.02% polysorbate 20, pH 6.0;
(4) 10 mM histidine-sulfate, 240 mM sucrose, 0.02% polysorbate 20 at pH 6.0.

Each formulation was filled into 3 cc FORMA VITRUM™ USP Type I glass vial sealed with FLUROTEC™ faced butyl rubber stoppers. Samples were stored at 30°C and 40°C and stability was evaluated for quality (CAC) and purity (SEC, IEC). The stability results showed that Pertuzumab in histidine-phosphate buffer degraded much faster than in other histidine buffers upon storage at 40°C (Fig. 8 and Fig. 9).

EXAMPLE 4

Pertuzumab was concentrated by ultrafiltration/diafiltration to various concentrations in the following buffers:
(1) 20 mM histidine-acetate, pH 6.0;
(2) 10 mM histidine-HCl, pH 6.0, and
(3) 10 mM histidine-sulfate, pH 6.0.

The turbidity of each formulation was measured before the filtration. The results, as shown in Fig. 10, demonstrated that Pertuzumab samples formulated in histidine-acetate and histidine-HCl had less amounts of insoluble aggregates than those in histidine-sulfate buffer.

EXAMPLE 5

Pertuzumab was formulated at 30 mg/mL in 20 mM histidine-acetate, 120 mM sucrose, 0.02 % polysorbate 20, pH 6.0. Pertuzmab was filled in 316L and HASTELLOY™ stainless steel miniature tanks. All samples were stored at -20°C and 5°C and evaluated for quality (CAC), purity (SEC, IEC) and strength (UV-Vis). The stability analyses showed that Pertuzumab was stable in this formulation upon storage at –20°C and 5°C for at least 3 months. The chloride free formulation is compatible with 316L and HASTELLOY™ stainless steel tank.

Table 4. Stability of Pertuzumab in Stainless Steel Tanks

Tanks	Temp (°C)	Time (Months)	CAC	UV Spec. (mg/mL)	SEC (% monomer)	IEC (% main peak)
		0	Pass[a]	29.0	99.8	67.9
316L	- 20	3	Pass	28.9	99.7	66.8
	5	3	Pass	28.7	99.7	66.8

HASTELLOY™	- 20	3	Pass	29.1	99.7	66.8
	5	3	Pass	28.8	99.7	67.7

[a] Pass for Color, Appearance and Clarity: Clear to slightly opalescent, colorless to pale yellow solution.

EXAMPLE 6

Pertuzumab was formulated using tangential flow filtration (TFF). The final formulation contains 20 mM histidine-acetate, 120 mM sucrose, 0.02 % polysorbate 20, pH 6.0 at protein concentration of 30 mg/mL. Samples were filled into a 20 Ml FORMA VITRUM™ USP Type I glass vial, capped with the 20 mm FLUROTEC™ faced butyl rubber stoppers, and sealed with aluminium flip-top caps. All samples were stored at –70°C, 5°C, 15°C, and stability was evaluated for quality (CAC), purity (SEC, IEC), strength (UV-Vis), and potency (Bioassay). The results showed that Pertuzumab is stable in this formulation upon storage at 5°C and 15°C for at least 3 months.

Table 5. Stability of Pertuzumab in glass vials

Temp (oC)	Time (Months)	CAC	UV Spec. (mg/mL)	SEC (% mononer)	IEC (% main peak)	Bioassay (% specific activity)
	0	Pass	29.2	99.8	64.1	83
-70	1	Pass	29.7	99.8	65.2	92
	3	Pass	30.7	99.8	67.0	93
5	3	Pass	30.4	99.7	67.2	90
15	1	Pass	29.7	99.7	64.4	78
	3	Pass	30.4	99.7	65.5	93

EXAMPLE 7

Pertuzumab was formulated at 100 mg/mL in the following buffer conditions:

(1) 10 mM histidine-HCl, pH 6.0;

(2) 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(3) 20 mM succinate at pH 6.0; and

(4) 20 mM succinate, 240 mM sucrose at pH 6.0.

Each formulation was added with different concentration of polysorbate 20. All samples were filled into 3 cc USP Type I glass vials and were agitated horizontally at 70 rpm at room temperature for up to 7 days. The stability of each sample was evaluated at 7 day time point for turbidity. The results demonstrated that the use of polysorbate 20 in the final formulation effectively prevented formation of insoluble aggregates. See Fig. 11.

EXAMPLE 8

Pertuzumab was prepared in the following formulations:

(1) 25 mg/mL Pertuzumab, 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(2) 50 mg/mL Pertuzumab, 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(3) 60 mg/mL Pertuzumab, 20 mM histidine-acetate, 120 mM sucrose, pH 6.0.

Various amounts of polysorbate 20 were added to each formulation. All samples were filled into 3 cc USP Type I glass vials, and agitated horizontally at 70 rpm at room temperature for up to 7 days. The physical stability of each sample was evaluated at 7 day time point for turbidity. The results demonstrated that the use of polysorbate 20 in histidine-HCl and sucrose formulation effectively prevented formation of insoluble particulates. The formulation containing histidine-acetate and sucrose appeared to have the same protective effect on protein as polysorbate 20. See Fig. 12.

EXAMPLE 9

Pertuzumab was formulated as follows:

(1) 100 mg/mL protein, 10 mM histidine-HCl, pH 6.0;

(2) 100 mg/mL protein, 20 mM succinate, pH 6.0;

(3) 60 mg/mL protein, 20 mM histidine-acetate, pH 6.0.

Each formulation was mixed with different amounts of sucrose. All samples were sterilely filled into 3 cc USP Type I glass vials. They were then frozen at –70°C and thawed at 5°C three times. The physical stability of each sample was determined after the three cycles of freezing and thawing. The results demonstrated that sucrose prevents soluble aggregate formation during the freeze-thawing process. See Fig. 13.

EXAMPLE 10

The preferred Pertuzumab formulation for therapeutic use consists essentially of 30mg/mL Pertuzumab in 20mM histidine acetate, 120mM sucrose, 0.02% polysorbate 20, at pH 6.0.

Compound	Concentration	Amount/L
Pertuzumab	30 mg/mL	30 g
L-Histidine MW=155.16g/mol	20 mM	3.10 g
Glacial Acetic Acid MW=60.05g/mol Density=1.05g/cm^3	11.6 mM	0.66 mL
Sucrose MW=342.3g/mol	120 mM	41.1 g
Polysorbate 20 Density=1.012g/cm^3	0.02% (w/v)	0.2 mL

MW: Molecular weight

420mg dose vial configuration:
Vial: 20 cc Formal Vitrum Type I glass
Stopper: 20mm DAIKYO GREY™, fluoro-resin laminated
Cap: 20mm flip top aluminum
Fill volume: 14.50 mL
Delivery: 14.0 mL Pertuzumab in normal saline IV bag.

1050mg dose vial configuration:
Vial: 50 cc Formal Vitrum Type I glass
Stopper: 20mm DAIKYO GREY™, fluoro-resin laminated
Cap: 20mm flip top aluminum
Fill volume: 36.0 mL
Delivery: 35.0 mL Pertuzumab in normal saline IV bag.

EXAMPLE 11

This example concerns another Pertuzumab formulation which has been used in Phase I and Phase II clinical trials. The composition consists of 25 mg/ml Pertuzumab, 10 mM Histidine-HCl buffer, 240 mM sucrose, 0.02% Polysorbate 20, pH 6.0.

Ingredient	Concentration
Pertuzumab	25 mg/ml
L-His HCl.H_2O (MW 209.6)	1.12 mg/ml (0.0125 M)
L-His(MW 155.2)	0.72 mgml (0.0099 M)
Sucrose (MW342.3)	82.15 mg/ml (0.240 M)
Polysorbate 20	0.2 mg/ml (0.02 %)

EXAMPLE 12

Cellular apoptosis is mediated by intrinsic and extrinsic pathways. Chemotherapy can cause cell damage and may trigger apoptosis by the intrinsic pathway in response to cellular damage. However, cancer cells often develop resistance to chemotherapy through mutations in the p53 tumor suppressor gene (Ashkenazi A. Targeting Death and Decoy Receptors of the Tumour-Necrosis Factor Superfamily. *Nature Reviews* 2:420-430 (2002)). Death receptors, such as DR4 and DR5, located on the surface of cells trigger apoptosis via the extrinsic pathway that does not involve p53. Agonistic molecules, such as Apo2L, bind to DR4 and DR5 receptors and activate caspases 8 and 10 through Fas-associated death domain. Caspase 8 and 10 then activate caspases 3, 6, and 7 to induce apoptosis. Molecular signaling of death receptors on tumor cells has therapeutic potential for the elimination of cancer cells that are resistant to conventional therapies and molecules, like Apo2L, are currently undergoing clinical evaluation.

"Apomab" is a full-length CHO derived humanized IgG1 constructed with a lamda light chain. It is an agonist antibody against DR5 that has been shown to induce apoptosis of various cancer cell lines. Preclinical studies using a murine tumor implant model have shown that Apomab has similar or improved tumor reduction compared to Apo2L. Apomab is being evaluated as an anti-cancer agent in the indications of advanced solid tumors and Non-Hodgkin's Lymphoma (NHL). The heavy and light chain amino acid sequences of Apomab used in these experiments are shown in Figs. 27 and 28.

Preparation of Antibody Formulations

Recombinantly produced Apomab had very dilute protein concentration and high pH. The material was concentrated to approximately 20 mg/mL and exchanged into 20 mM sodium acetate, pH 5.0 buffer using a Millipore Labscale tangential flow filtration (TFF) system with MILLIPORE PELLICON™ XL, PLCGC10, 50 cm membrane. Apomab samples were formulated into various buffer systems covering pH range from 4.0 to 7.0 using sodium acetate, histidine acetate, and sodium phosphate without trehalose and TWEEN 20® using dialysis with a 10,000 Da molecular weight cut off membrane (Pierce, Inc). Trehalose at 240 mM was added in the last dialysis. After dialysis, 0.02% TWEEN 20™ was added to the formulation and the samples were filtered with 0.22 µm filters (Millipore, Inc.). A 0.5 mL volume of Apomab was filled into sterile 3 cc glass vials (Forma Vitrum, Inc.) and sealed with 13 mm stoppers (Daikyo, Inc). Protein stability was evaluated at -70°C, 5°C, 30°C, and 40°C with storage for up to 3 months.

Stability of Apomab Formulation

For drug product stability testing, Apomab formulated bulk filled into 5 cc FORMA VITRUM® glass vials were formulated. Vials were filled with 5.5 mL of formulated antibody, fitted with 20 mm DAIKYO® stoppers, and stored at -70°C, 5°C, 30°C, and 40°C in the upright position.

For drug substance stability testing, Apomab formulated bulk was sterile filtered through a 0.22 µm filter and 10 mL was filled into autoclaved 20cc 316L stainless steel mini-tanks. The tanks were placed upright at -20°C and 5°C. A 1 mL aliquot was aseptically removed from the mini-tanks at specified time intervals to assess protein quality. The control vials were 1 mL aliquots in 3 cc glass vials stored at -20°C.

Color, Appearance, and Clarity

The clarity, appearance, and color of the samples were visually assessed under white fluorescent light using a light inspection station with black and white background. For analysis of the drug substance, mini-tank samples were transferred to a 3 cc glass vial for inspection.

pH

pH was measured at room temperature with THERMO ORION SURE-FLOW ROSS™ semi-micro pH electrode for measuring buffers or THERMO ORION GLS™ combination micro pH electrode for measuring protein pH screening samples, a Beckman microelectrode probe for Toxicology stability samples. The METERLAB™ pHM240 pH/Ion meter (Radiometer Analytical) was calibrated every day with buffer standards (EM Science) at pH 7 and pH 4.

Concentration

Protein concentration was determined by ultraviolet absorption spectroscopy using an AGILENT 8453™ spectrophotometer. The samples were diluted with appropriate formulation buffer blanks to give an absorbance from 0.5 to 1.0. The instrument was blanked with the diluent solution and the spectrum was scanned from 240 to 500 nm. The absorbance value at 320 nm was subtracted from the absorbance at 279 nm to correct for offset and light scattering. The protein concentrations were calculated by the following equation:

$$\text{Conc. (mg/mL)} = \frac{(A279 - A320) \times \text{dilution factor}}{\text{absorptivity coefficient in } cm^{-1}(mg/mL)^{-1}}$$

The absorptivity coefficient based on sequence was initially determined to be 1.32 $cm^{-1}(mg/mL)^{-1}$ and this value was used for the pH screening studies. A later value of 1.7 $cm^{-1}(mg/mL)^{-1}$ was determined by amino acid analysis and proteolysis methods and this value was used for the stability analysis of Apomab used in Toxicology studies.

Ion-Exchange Chromatography

Ion exchange chromatography was carried out on an 1100 series HPLC (Agilent Technologies, Inc.) equipped with a diode array detector. Chromatography was carried out on a PROPAC WCX-10™ (Dionex) column (4 x 250 mm) at a flow rate of 0.5 mL/min and with column temperature at 40°C. Mobile phase A was

25 mM sodium phosphate, pH 6.5. Mobile phase B was 100 mM sodium chloride in the same buffer as mobile phase A. The column was equilibrated with 100% mobile phase A. For pH screening samples an amount of 20 mg of Apomab was loaded onto the column and the absorbance was monitored at 214 nm. Protein was eluted from the column with the following gradient:

Time (min)	%A	%B
0	100	0
50	0	100
51	100	0
70	100	0

For stability analysis of material used in the Toxicology studies an amount of 30 mg of Apomab was loaded onto the column and the absorbance was monitored at 280 nm. Protein was eluted from the column with the following gradient:

Gradient:	Time (min)	%A	%B
	0	100	0
	40.0	40	60
	41.0	0	100
	45.0	0	100
	45.1	100	0
	60.0	100	0

Size-Exclusion Chromatography

Size exclusion chromatography was carried out on an 1100 series HPLC (Agilent Technologies, Inc.) equipped with a diode array detector. An amount of 50 µg Apomab was loaded onto a TSK Gel 3000SWXL™ (7.8 x 300 mm) column and run at a flow rate of 0.9 mL/min for 20 minutes for pH screening samples and 0.5 mL/min for 30 minutes for Toxicology stability samples with 0.20 M potassium phosphate, 0.25 M potassium chloride, pH 6.2 as a mobile phase. Absorbance was monitored at 280 nm.

Potency

The purpose of the potency bioassay was to measure the ability of Apomab to kill Colo205 cells using ALAMARBLUE™. Colo205 is a colon carcinoma cell line, which expresses both DR5 and DR4 death receptors. This assay incorporates a fluorometric/colorimetric growth indicator based on detection of metabolic activity. ALAMARBLUE™ is a redox dye that is blue and non-fluorescent in oxidized state. The intracellular metabolic reduction converts it into a red color that is also fluorescent. The changes in color and fluorescence are proportional to the metabolic activity and number of living cells. The signal decreased when cells die. Apomab was diluted in medium with anti-Fc and then Colo 205 cells were added to Apomab samples and incubate at 37°C for 48 hours. ALAMARBLUE™ is added for the last 2-3 hours. The plate was read at 530 nm excitation and 590 nm emission to get relative fluorescence units (RFU). The data were analyzed by KALEIDAGRAPH™. A dilution curve of killing was generated.

RESULTS

Formulation pH Screen Study

The effect of pH on antibody stability was studied using Apomab produced from an unamplified stable cell line. For this analysis, Apomab was formulated at 20 mg/mL antibody in 20 mM sodium acetate buffer at pH 4.0, 4.5, 5.0, 5.5; 20 mM histidine acetate buffer at pH 6.0 and 6.5; and 20 mM sodium phosphate buffer at pH 7.0. All of the formulations contained 240 mM trehalose and 0.02% TWEEN 20®. The formulations were stored for up to 3 months at temperatures of -70°C, 5°C, 30°C, and 40°C and protein stability was determined by various analytical assays, including CAC, pH, concentration, SEC and IEC. No significant changes in CAC, pH or protein concentration were observed during storage of the samples.

Analysis of the samples by SEC showed that no significant changes occurred during storage at 5°C and -70°C. However, degradation observed as the formation of antibody fragments and soluble aggregates occurred during storage at 30°C and 40°C (Fig. 20). To compare the formulations, antibody monomer kinetics during storage was monitored and the first-order rate constants were calculated. The obtained pH rate profile for the loss in antibody monomer is shown in Fig. 21. The optimal condition for the stability of antibody monomer was obtained by formulating in histidine acetate buffer at pH 6.0.

Apomab charge heterogeneity was monitored by IEC. No significant changes in the IEC profile occurred during storage at 5°C and -70°C. However, degradation observed as the formation of acidic or basic variants occurred depending on the formulation (Fig. 22). In general, increased basic variants were formed at lower formulation pH and more acidic variants were formed at higher formulation pH. To compare the formulations, IEC main peak kinetics was monitored during storage and the first-order rate constants were calculated. The obtained pH rate profile for the loss in IEC main peak is shown in Fig. 23. The rate constants observed by IEC were approximately 10 fold higher than those from SEC (Fig. 21). Therefore, the loss in IEC main peak was the primary degradation of the antibody that will ultimately limit the product shelf life. Furthermore, as observed by SEC, optimal antibody stability to stabilize IEC main peak was obtained by formulating in histidine acetate buffer at pH 6.0.

Following the analysis of pH screening data described above, an Apomab formulation was selected that comprised 20 mg/mL antibody in 20 mM histidine acetate, 240 mM trehalose, 0.02% polysorbate 20, pH 6.0. For the drug product, the vial configuration consisted of 5.5 mL fill in a 5 cc FORMA VITRUM™ vial with a 20 mM DAIKYO™ West stopper. Apomab was stored in stainless steel tanks.

The stability of Apomab Drug Product was evaluated in the 5cc glass vial configuration described above. Vials were stored at -70°C (controls), 5°C, 30°C, and 40°C. Samples were pulled at specific time intervals and analyzed by the following assays: color, appearance, clarity (CAC), pH, protein concentration, SEC, IEC, and potency. The results from these assays are shown in Table 6 for samples stored at -70°C and 5°C and Table 7 for samples stored at 30°C and 40°C.

Table 6. Stability Data for Apomab Stored at -70°C and 5°C

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ±0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 - 140%*
NA	T=0	Clear	Colorless	5.9	20.2	99.8	63	94
-70	1 month	Clear	Colorless	6.0	20.5	99.8	63	86
-70	2 month	Clear	Colorless	6.0	20.4	99.7	64	91
-70	3 month	Clear	Colorless	6.0	20.5	99.7	63	83
-70	6 month	Clear	Colorless	6.0	20.4	99.7	64	85
-70	9 month	Clear	Colorless	6.0	20.4	99.8	65	89
-70	12 month	Clear	Colorless	6.0	20.8	99.7	63	107
5	1 month	Clear	Colorless	6.0	20.5	99.7	63	89
5	2 month	Clear	Colorless	6.0	20.4	99.7	64	99
5	3 month	Clear	Colorless	6.0	20.6	99.7	63	84
5	6 month	Clear	Colorless	6.0	20.5	99.7	64	93
5	9 month	Clear	Colorless	6.0	20.6	99.7	64	88
5	12 month	Clear	Colorless	6.0	20.7	99.8	64	106

Table 7. Stability Data for Apomab Stored at 30°C and 40°C

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ± 0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 - 140%*
30	1 month	Clear	Colorless	6.0	20.6	98.2	59	91
30	2 month	Clear	Colorless	6.0	20.3	97.4	54	80
30	3 month	Clear	Colorless	6.0	20.6	97.2	49	74
30	6 month	Clear	Colorless	6.0	20.2	94.1	37	51
30	9 month	Clear	Slightly yellow	6.0	20.4	93.2	31	55
30	12 month	Clear	Slightly yellow	6.0	20.6	91.6	25	59
40	1 month	Clear	Colorless	6.0	20.4	96.6	44	79
40	2 month	Clear	Colorless	6.0	20.0	93.7	31	64
40	3 month	Clear	Slightly yellow	5.9	20.3	91.5	22	53
40	6 month	Clear	Slightly yellow	6.0	20.2	83.9	NT	28
40	9 month	Clear	Yellow	5.9	20.3	78.6	NT	25
40	12 month	Clear	Yellow	5.9	20.5	71.4	NT	31

NT = not quantitated

No change in protein quality was observed after twelve months storage at -70°C and 5°C. For instance, the pH remained at 6.0 ± 0.3, Apomab appeared as a clear and colorless liquid, the protein concentration remained at 20.0 ± 2.0 mg/mL, and % monomer was unchanged. Furthermore, there was no significant change in % IEC main peak and % specific activity determined by the cell-killing potency assay was within the assay precision of 60 % to 140 % specific activity. The results showed that Apomab stored in 5 cc glass vials was stable for at least 12 months at 5°C.

Table 7 shows that changes in protein quality occurred at 30°C and 40°C. SEC showed a decrease in % monomer with a rise primarily in fragment species. Aggregates increase as well at higher temperature, but the rate was much slower. However, the aggregates increase significantly after 6 months at 40°C. IEC % main peak decreased with a corresponding increase in acidic variants. Basic peaks decreased slightly after 2 months at 40°C and 9 months at 30°C. After six months of storage at 40°C, degradation occurred to an extent that IEC main peak could no longer be integrated. The cell killing bioassay showed loss of % specific activity at higher temperature with longer storage time. Protein concentration and pH were unchanged. The solution becomes slightly yellow after 3 months at 40°C and 9 months at 30°C and becomes yellow after 9 months at 40°C.

Drug Substance Stability

Freeze-thaw stability data for drug substance are shown in Table 8.

Table 8. Freeze-Thaw Stability Data for Apomab Filled in Miniature Stainless Steel Tanks

Temp (°C) (Frozen/thaw)	Freeze-Thaw Cycle No.	Clarity	Color	pH	Concentration (mg/m L)	SEC (% Monomer)
Acceptance Criteria:		*Report*	*Report*	*6.0±0.3*	*20.0±2.0*	*≥95%*
Control (unfrozen)	0	Clear	Colorless	6.0	20.9	99.6
-20/25	1	Clear	Colorless	6.0	20.8	99.6
-20/25	2	Clear	Colorless	6.0	20.8	99.6
-20/25	3	Clear	Colorless	6.0	20.9	99.6

Table 9. Stability Data for Apomab Filled in Miniature Stainless Steel Tanks

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ± 0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 – 140%*
NA	T=0	Clear	Colorless	5.9	20.0	99.7	63	88
-20	1 month	Clear	Colorless	6.0	20.6	99.7	63	107
-20	3 month	Clear	Colorless	6.0	20.6	99.7	63	82
-20	6 month	Clear	Colorless	6.0	20.3	99.7	64	92
-20	9 month	Clear	Colorless	6.0	20.6	99.7	64	92
-20	12 month	Clear	Colorless	6.0	21.2	99.7	65	94
5	1 month	Clear	Colorless	6.0	20.5	99.7	62	95
5	3 month	Clear	Colorless	6.0	20.7	99.6	62	71
5	6 month	Clear	Colorless	6.0	20.4	99.5	62	84
5	9 month	Clear	Colorless	6.0	20.8	99.4	61	84
5	12 month	Clear	Colorless	6.0	21.3	99.2	59	82

No significant changes in the chemical characteristics of the protein were observed after being frozen at -20°C for at least 15 hours and thawed at ambient temperature three times. For example, Apomab appeared as a clear and colorless liquid, the pH remained at 6.0 ± 0.3, and the SEC monomer peak percentage was unchanged.

Apomab stability in stainless steel containers was evaluated at -20°C and 5°C (Table 9).

Samples were aseptically pulled from the mini-tanks at specific intervals and analyzed.

Apomab showed no change in protein quality at 5°C by pH, CAC, protein concentration and % main peak by IEC but lost 0.1% monomer by SEC every 3 months. Decreased potency was observed during storage at 5°C for

3 months. However, the potency of the sample increased again at the 6 and 9 month timepoints. Therefore, the observed potency difference at the 3 month timepoint was attributed to assay variation. Apomab showed no change in protein quality at -20°C by pH, CAC, protein concentration, % monomer by SEC, % main peak by IEC, and no significant change in potency. The stability data show that Apomab is stable for at least 1 year at -20°C and three months at 5°C.

CONCLUSION

Formulation screening studies were performed to select a formulation for Apomab. A pH screen covering the pH range 4.0 to 7.0 using sodium acetate, histidine acetate, and sodium phosphate as buffers with 240 mM trehalose dihydrate and 0.02% polysorbate 20 showed that Apomab is most stable in solution at pH 6.0. Therefore, a formulation consisting of 20 mM histidine acetate, 240 mM trehalose, 0.02% polysorbate 2, pH 6.0 was developed and demonstrated experimentally to be stable. Using this formulation, Apomab was shown to be stable for at least 12 months at 5°C. Furthermore, Apomab was shown to be stable for at least 12 months at -20°C and three months at 5°C when stored in 316L stainless steel containers. Apomab was also shown to be stable when subjected to up to 3 freeze/thaw cycles.

WHAT IS CLAIMED IS:

1. A stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5.

2. The formulation of claim 1 wherein the pH is from 5.8 to 6.2.

3. The formulation of claim 1 wherein the histidine-acetate buffer concentration is from about 1mM to about 200mM.

4. The formulation of claim 3 wherein the histidine-acetate buffer concentration is from about 10mM to about 40mM.

5. The formulation of claim 1wherein the antibody concentration is from about 10mg/mL to about 250mg/mL.

6. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 20mg/mL to about 40mg/mL.

7. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 80mg/mL to about 250mg/mL.

8. The formulation of claim 1 further comprising saccharide.

9. The formulation of claim 8 wherein the saccharide is a disaccharide.

10. The formulation of claim 8 wherein the saccharide is trehalose.

11. The formulation of claim 8 wherein the saccharide is sucrose.

12. The formulation of claim 8 wherein the saccharide concentration is from about 10mM to about 1M.

13. The formulation of claim 12 wherein the saccharide concentration is from about 60mM to about 250mM.

14. The formulation of claim 1 further comprising surfactant.

15. The formulation of claim 14 wherein the surfactant is polysorbate.

16. The formulation of claim 15 wherein the surfactant is polysorbate 20.

17. The formulation of claim 14 wherein the surfactant concentration is from about 0.0001% to about 1.0%.

18. The formulation of claim 17 wherein the surfactant concentration is from about 0.01% to about 0.1%.

19. The formulation of claim 1 wherein the monoclonal antibody is a full length antibody.

20. The formulation of claim 19 wherein the monoclonal antibody is an IgG1 antibody.

21. The formulation of claim 1 wherein the monoclonal antibody is a humanized antibody.

22. The formulation of claim 1 wherein the monoclonal antibody is an antibody fragment comprising an antigen-binding region.

23. The formulation of claim 22 wherein the antibody fragment is a Fab or F(ab')2 fragment.

24. The formulation of claim 1 which is sterile.

25. The formulation of claim 1 wherein the monoclonal antibody binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

26. The formulation of claim 25 wherein the antigen is CD20 and the monoclonal antibody is humanized 2H7.

27. The formulation of claim 25 wherein the antigen is VEGF and the monoclonal antibody is Bevacizumab.

28. The formulation of claim 1 wherein the monoclonal antibody is susceptible to deamidation or aggregation.

29. The formulation of claim 1 which is stable upon storage at about 40°C for at least 4 weeks

30. The formulation of claim 1 which is stable upon storage at about 5°C or about 15°C for at least 3 months.

31. The formulation of claim 1 which is stable upon storage at about -20°C for at least 3 months.

32. The formulation of claim 1 which is stable upon freezing and thawing.

33. The formulation of claim 1 which is aqueous.

34. The formulation of claim 1 which is frozen.

35. The formulation of claim 1 which is not lyophilized and has not been subjected to prior lyophilization.

36. The formulation of claim 35 which is aqueous and is administered to a subject.

37. The formulation of claim 36 wherein the formulation is for intravenous (IV), subcutaneous (SQ) or intramuscular (IM) administration.

38. The formulation of claim 37 which is for IV administration and the antibody concentration is from about 20mg/mL to about 40mg/mL.

39. The formulation of claim 37 which is for SQ administration and the antibody concentration is from about 80mg/mL to about 250mg/mL.

40. A vial with a stopper pierceable by a syringe comprising the formulation of claim 1 inside the vial.

41. The vial of claim 40 which is stored at about 2-8°C.

42. The vial of claim 40 which is a 20cc or 50cc vial.

43. A stainless steel tank comprising the formulation of claim 1 inside the tank.

44. The tank of claim 43 wherein the formulation therein is frozen.

45. A method of treating a disease or disorder in a subject comprising administering the formulation of claim 1 to a subject in an amount effective to treat the disease or disorder.

46. A pharmaceutical formulation comprising:

(a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL;

(b) histidine-acetate buffer, pH 5.5 to 6.5;

(c) sacoharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and

(d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

47. A method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5

48. The method of claim 47 comprising evaluating any antibody deamidation or aggregation before and after the antibody is formulated.

49. A pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

50. The formulation of claim 49 wherein the buffer is histidine-acetate.

51. The formulation of claim 49 wherein the HER2 antibody comprises the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.

52. The formulation of claim 51 wherein the HER2 antibody comprises a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

53. The formulation of claim 49 wherein the pH of the formulation is from about 5.8 to about 6.2.

54. The formulation of claim 49 wherein the antibody binds to the junction between domains I, II and III of HER2.

55. The formulation of claim 49 wherein the antibody is a full length antibody.

56. The formulation of claim 49 wherein the antibody concentration is from about 20mg/mL to about 40mg/mL.

57. A pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

58. The formulation of claim 57 comprising about 30mg/mL Pertuzumab, about 20mM histidine-acetate, about 120mM sucrose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

59. A vial with a stopper pierceable by a syringe comprising the formulation of claim 49.

60. A stainless steel tank comprising the formulation of claim 49 in the tank.

61. A method of treating HER2-expressing cancer in a subject, comprising administering the pharmaceutical formulation of claim 49 to the subject in an amount effective to treat the cancer.

62. The method of claim 61 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

63. A method of making a pharmaceutical formulation comprising:

(a) preparing the formulation of claim 1; and

(b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

64. A pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

65. The formulation of claim 64 wherein the buffer is histidine-acetate.

66. The formulation of claim 64 wherein the DR5 antibody is an agonist antibody.

67. The formulation of claim 64 wherein the DR5 antibody is Apomab.

68. The formulation of claim 67 wherein the DR5 antibody comprises the heavy chain amino acid sequence of SEQ ID No. 51, and light chain amino acid sequence of SEQ ID No. 52.

69. The formulation of claim 64 wherein the pH of the formulation is from about 5.8 to about 6.2.

70. The formulation of claim 64 wherein the antibody is a full length antibody.

71. The formulation of claim 64 wherein the antibody concentration is from about 10mg/mL to about 30mg/mL.

72. A pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

73. The formulation of claim 72 comprising about 20mg/mL Apomab, about 20mM histidine acetate, about 240mM trehalose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

74. A vial with a stopper pierceable by a syringe comprising the formulation of claim 64.

75. A stainless steel tank comprising the formulation of claim 64 in the tank.

76. A method of treating cancer in a subject, comprising administering the pharmaceutical formulation of claim 64 to the subject in an amount effective to treat the cancer.

77. The method of claim 76 wherein the cancer is a solid tumor.

78. The method of claim 76 wherein the cancer is non-Hodgkin's lymphoma.

79. The method of claim 76 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

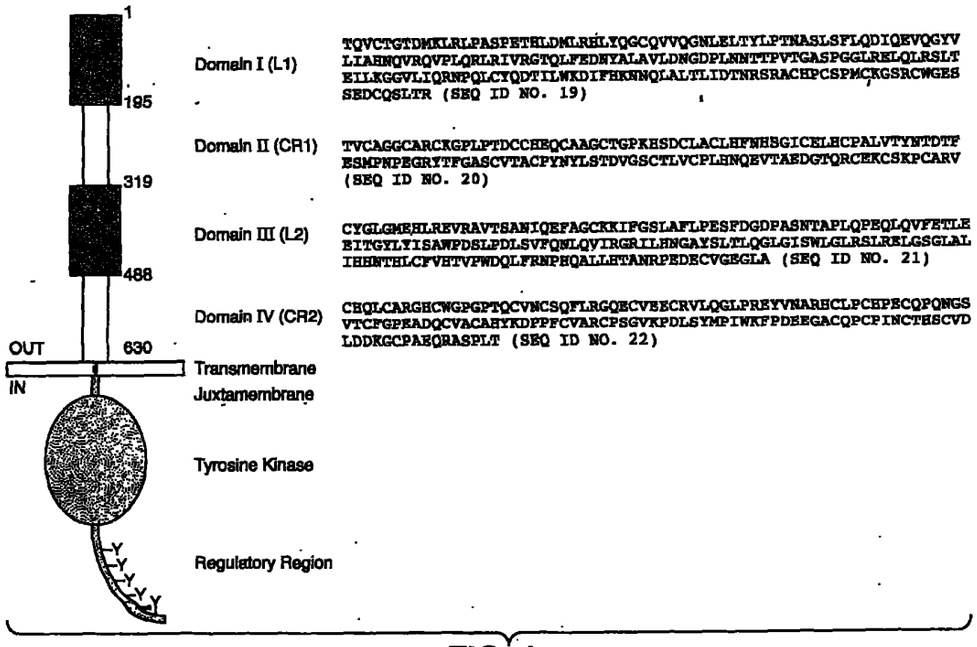


FIG. 1

WO 2006/044908

PCT/US2005/037471

Variable Light

```
                  10          20          30          40
2C4      DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
          **    **** *       *                    *
574      DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                  *   ** ***
hum κI   DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                       50          60          70          80
2C4      GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
          **                       *  *          *    * *
574      GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                    * *****
hum κI   GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                    90          100
2C4      EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
           * *                    *   *
574      EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                       *** *
hum κI   EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

FIG. 2A

Variable Heavy

```
                  10          20          30          40
2C4      EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
             **   **  *  * ***  *                  * *
574      EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                     ** * *
hum III  EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                       50  a       60          70          80
2C4      HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                      *** *    **** *
574      PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                    ****** *** ****       * * *
hum III  PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                  abc       90          100ab       110
2C4      ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS (SEQ ID NO:2)
         ***  **                                  **
574      QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                           ********
hum III  QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

FIG. 2B

Amino Acid Sequence for Pertuzumab Light Chain

```
1         10        20        30        40        50        60
DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPS

          70        80        90        100       110       120
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPP

          130       140       150       160       170       180
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

          190       200       210
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

FIG. 3A

Amino Acid Sequence for Pertuzumab Heavy Chain

```
1         10        20        30        40        50        60
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIY

          70        80        90        100       110       120
NQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSA

          130       140       150       160       170       180
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG

          190       200       210       220       230       240
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP

          250       260       270       280       290       300
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

          310       320       330       340       350       360
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

          370       380       390       400       410       420
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ

          430       440       448
QGNVFSCSVMHEALHNHYTQKSLSLSPG
```

FIG. 3B

```
1                               15                              30                              45
M G W S C I I L F L V A T A T G V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S

46                              60                              75                              90
Q D V S I G V A W Y Q Q K P G K A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F

91                              105                             120                             135
T L T I S S L Q P E D F A T Y Y C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F

136                             150                             165                             180
I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E

181                             195                             210                             225
S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T

226         233
K S F N R G E C   (SEQ ID NO. 17)
```

FIG. 4A

```
1                                15                                30                                45
M G W S C I I L F L V A T A T G V H S E V Q L V E S G G G L V Q P G G S L R L S C A A S G

46                               60                                75                                90
F T F T D Y T M D W V R Q A P G K G L E W V A D V N P N S G G S I Y N Q R F K G R F T L S

91                               105                               120                               135
V D R S K N T L Y L Q M N S L R A E D T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T

136                              150                               165                               180
V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N

181                              195                               210                               225
S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H

226                              240                               255                               270
K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D

271                              285                               300                               315
T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E

316                              330                               345                               360
Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A

361                              375                               390                               405
K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N

406                              420                               435                               450
G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H

451                              465
E A L H N H Y T Q K S L S L S P G   (SEQ ID NO. 18)
```

FIG. 4B

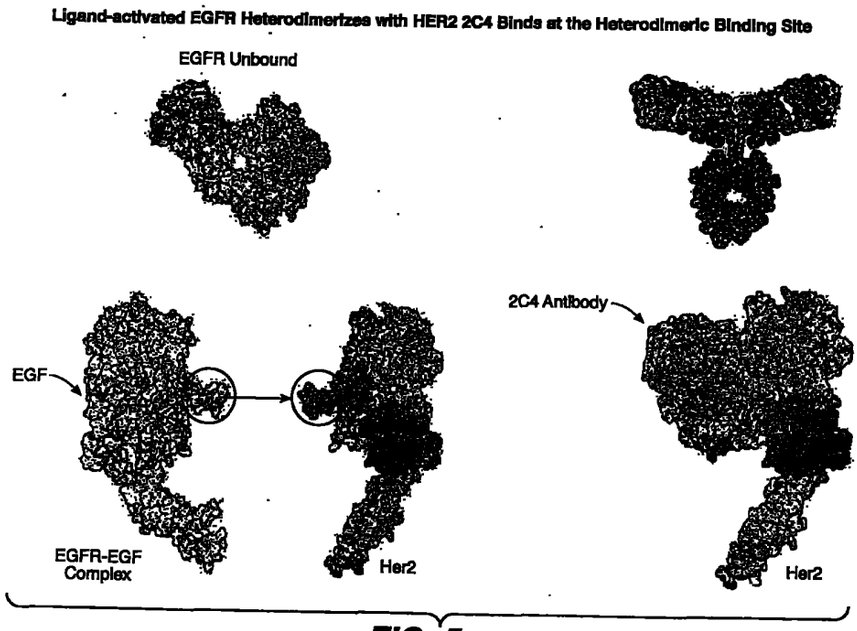

Ligand-activated EGFR Heterodimerizes with HER2 2C4 Binds at the Heterodimeric Binding Site
EGFR Unbound
EGF
EGFR-EGF Complex
Her2
2C4 Antibody
Her2

FIG. 5

Coupling of HER2/3 to the MAPK and Akt Pathways
HER3
HER2
HRG
Survival
Akt
PDK
PI3K
p85
IKK
Akt
Caspase 9
GSK
FKHR
Bad
Proliferation
Ras
Raf
MEK
MAPK
c-Myc
ERK
p90RSK

FIG. 6

Trastuzumab
Herceptin



- Binds in IV near JM.
- Protects against receptor shedding
- Moderately affects receptor down-modulation
- Slight effect on HER2's role as a coreceptor

Pertuzumab
Omnitarg



- Binds in II at dimerization interface
- Does not prevent receptor shedding
- Moderately affects receptor down-modulation
- Major effect on HER2's role as a coreceptor

FIG. 7

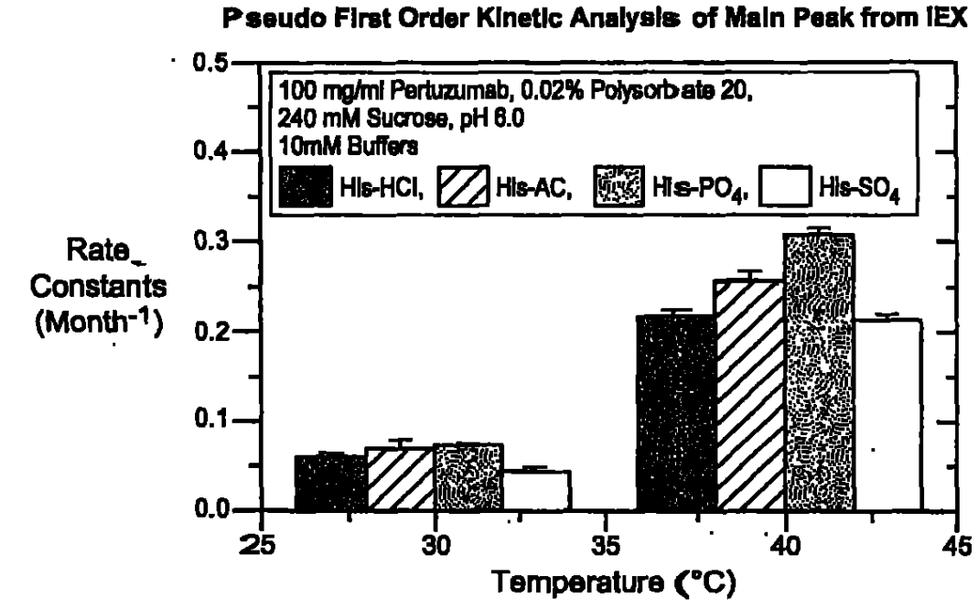

Pseudo First Order Kinetic Analysis of Main Peak from IEX
100 mg/ml Pertuzumab, 0.02% Polysorbate 20,
240 mM Sucrose, pH 6.0
10mM Buffers
His-HCl, His-AC, His-PO4, His-SO4
Rate Constants (Month-1)
0.5
0.4
0.3
0.2
0.1
0.0
25
30
35
40
45
Temperature (°C)

FIG. 8

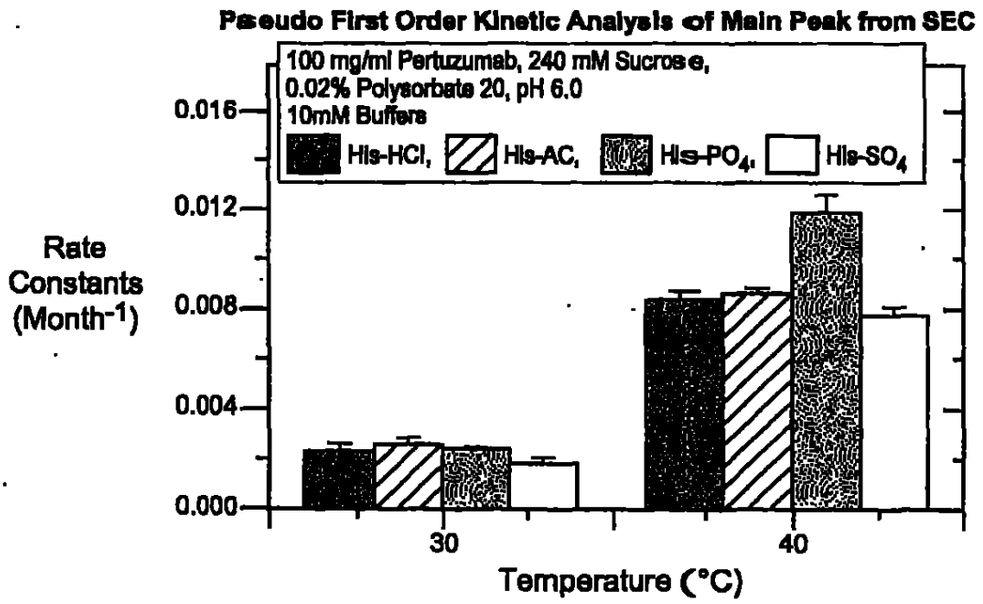

Pseudo First Order Kinetic Analysis of Main Peak from SEC
100 mg/ml Pertuzumab, 240 mM Sucrose,
0.02% Polysorbate 20, pH 6.0
10mM Buffers
His-HCl, His-AC, His-PO4, His-SO4
Rate Constants (Month-1)
0.016
0.012
0.008
0.004
0.000
30
40
Temperature (°C)

FIG. 9

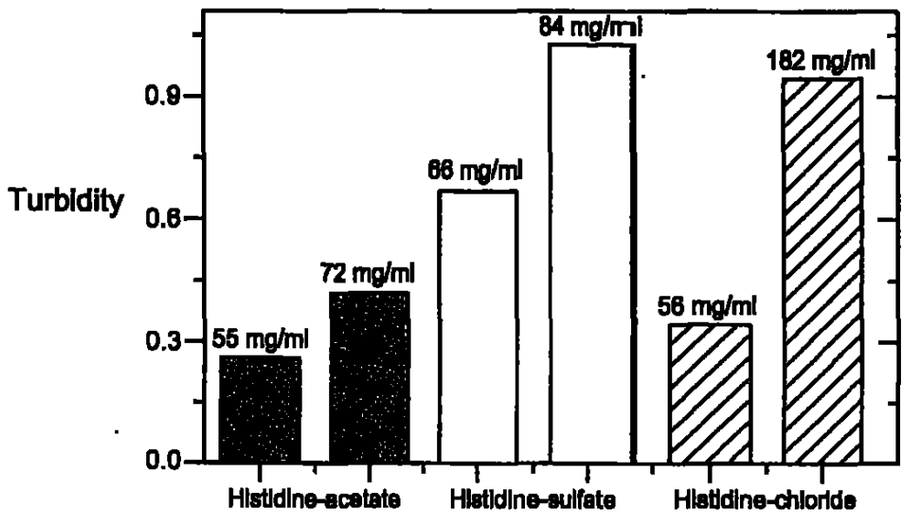

Turbidity
0.0
0.3
0.6
0.9
55 mg/ml
72 mg/ml
66 mg/ml
84 mg/ml
56 mg/ml
182 mg/ml
Histidine-acetate
Histidine-sulfate
Histidine-chloride

FIG. 10

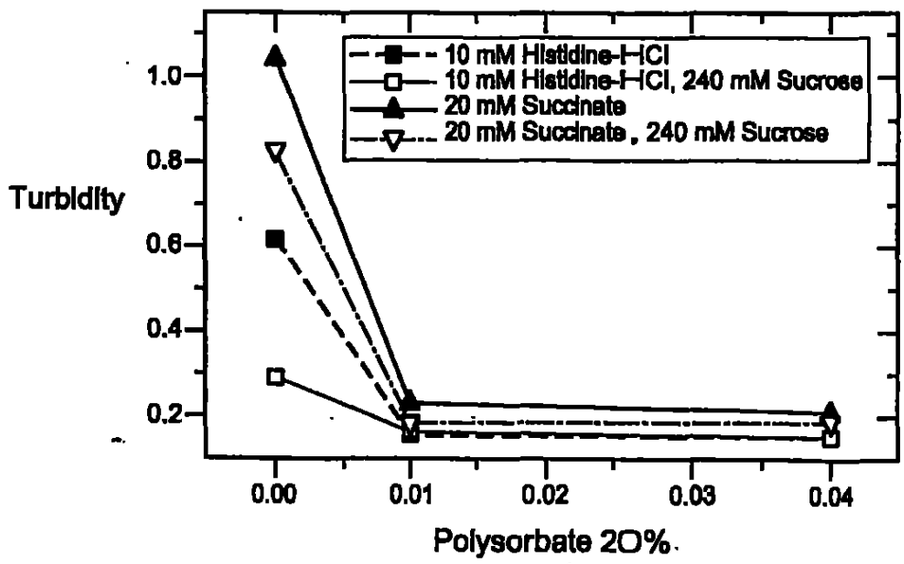

10 mM Histidine-HCl
10 mM Histidine-HCl, 240 mM Sucrose
20 mM Succinate
20 mM Succinate , 240 mM Sucrose
Turbidity
0.2
0.4
0.6
0.8
1.0
0.00
0.01
0.02
0.03
0.04
Polysorbate 20%

FIG. 11

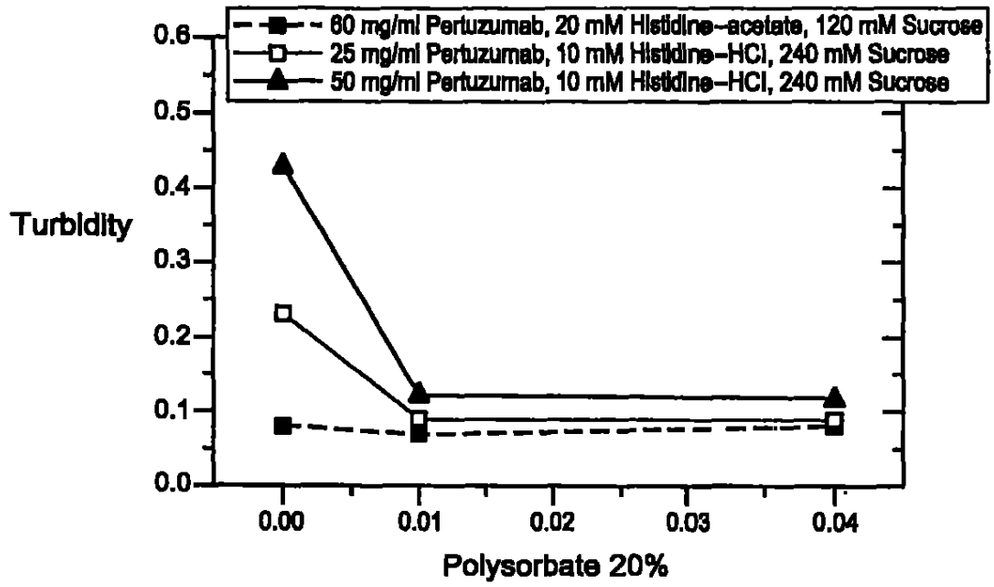

60 mg/ml Pertuzumab, 20 mM Histidine-acetate, 120 mM Sucrose
25 mg/ml Pertuzumab, 10 mM Histidine-HCl, 240 mM Sucrose
50 mg/ml Pertuzumab, 10 mM Histidine-HCl, 240 mM Sucrose
Turbidity
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.01
0.02
0.03
0.04
Polysorbate 20%

FIG. 12

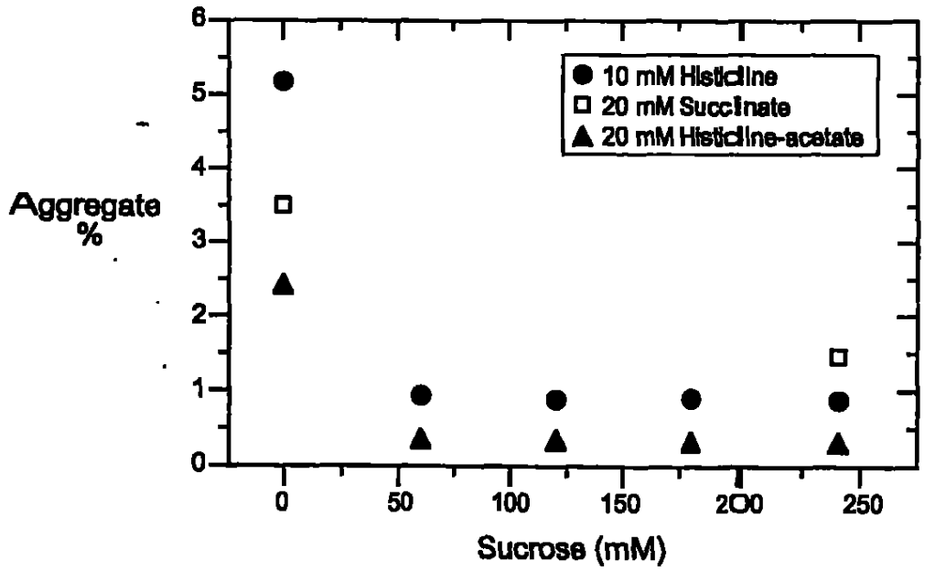

10 mM Histidine
20 mM Succinate
20 mM Histidine-acetate
Aggregate %
6
5
4
3
2
1
0
0
50
100
150
200
250
Sucrose (mM)

FIG. 13

Light Chain

```
1                  15                  30                  45
D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K

46                 60                  75                  90
L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q

91                 105                 120                 135
H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L

136                150                 165                 180
L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T

181                195                 210  214
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG. 14A

Heavy Chain

```
1                               15                              30                              45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L

46                              60                              75                              90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D

91                              105.                            120                             135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S

136                             150                             165                             180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

181                             195                             210                             225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K

226                             240                             255                             270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S

271                             285                             300                             315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D

316                             330                             345                             360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E

361                             375                             390                             405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

406                             420                             435                             449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
```

FIG. 14B

```
1                                 15                                30                                45
V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I G V A W Y Q Q K P G K

46                                60                                75                                90
A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y

91                                105                               120                               135
C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V

136                               150                               165                               180
V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S

181                               195                               210                 217
T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C   (SEQ ID NO. 23)
```


FIG. 15A

```
1                                15                                30                                45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T D Y T M D W V R Q A P G K G L

46                               60                                75                                90
E W V A D V N P N S G G S I Y N Q R F K G R F T L S V D R S K N T L Y L Q M N S L R A E D

91                               105                               120                               135
T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S K

136                              150                               165                               180
S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G

181                              195                               210                               225
L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K T

226                              240                               255                               270
H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H

271                              285                               300                               315
E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W

316                              330                               345                               360
L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M

361                              375                               390                               405
T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S

406                              420                               435                               449
F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
```

(SEQ ID NO. 24)

FIG. 15B

FIG. 16A

Oligosaccharide Structures Commonly Observed in IgG Antibodies

Structures	Abbreviation	Mass
Manα(1–>6) Manα(1–>3) > Manα(1–>6) Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	Man5	1235
GlcNAcβ(1–>2) { Manα(1–>6) Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G-1	1260
GlcNAcβ(1–>2)Manα(1–>6) GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	G0-F	1317
Manα(1–>6) Manα(1–>3) > Manα(1–>6) Manα(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	Man6	1398

Oligosaccharide Structures Commonly Observed in IgG Antibodies

Structures	Abbreviation	Mass
Galβ(1–>4)GlcNAcβ(1–>2){Manα(1–>6), Manα(1–>3)} > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G1-1	1423
GlcNAcβ(1–>2)Manα(1–>6), GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G0	1463
Galβ(1–>4)GlcNAcβ(1–>2)Manα(1–>6), GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G1 (1-6)	1626
GlcNAcβ(1–>2)Manα(1–>6), Galβ(1–>4)GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G1 (1-3)	1626
Galβ(1–>4)GlcNAcβ(1–>2)Manα(1–>6), Galβ(1–>4)GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) on GlcNAc)	G2	1788

Masses shown in this figure correspond to the $(M+Na)^+$ values.

FIG. 16B

Anti-IgE Antibodies: Light Chain (V_L and C_L Domains)

```
                10        20        30        40        50        60        70        80
E25     DIQLTQSPSS LSASVGDRVT ITC[RASQSVD YDGDSYMN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
E26     DIQLTQSPSS LSASVGDRVT ITC[RASKPVD GEGDSYLN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
HAE1    DIQLTQSPSS LSASVGDRVT ITC[RASKPVD GEGDSYLN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
Hu-901  DI[]LTQSP[S] LS[]A[S]G[D]R[V]T [I]C RASQS[G] TNIH---- WY QQKPG[K]AP[K]L LI[Y] [Y]AS[E]S[I]S  G[]PSRFSGSG SGTDFTLTIS

                90        100       110     C_L starts
E25     SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
E26     SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
HAE1    SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
Hu-901  [S]L[Q]PEDFA[T]Y YC QQS[D]SW[T]  T FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD

E25     SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 37
E26     SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 38
HAE1    SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 39
Hu-901  SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 40
```

FIG. 17A

Anti-IgE Antibodies: Heavy Chain (V_H and C_H Domains)

```
                10        20        30        40        50 a      60        70        80        90
E25     EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWNW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
E26     EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWNW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
HAE1    EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWNW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
Hu-901  [V]VQLV[Q]SG[AE] [VK]K[PG][AS][VK]V SC[KA]SGYTF- S [MH]GLEW [V]RQ APG[R]GLEWVG [EISPGTFTTNY NEKFKN] K[AT] T[TA][TA]D[KT]S[TA]Y [MEL]SSLR[SE]D

                100       110ab       120 C_H starts
E25     TAVYYCAR[GS HYFGHWHFAV]  WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
E26     TAVYYCAR[GS HYFGHWHFAV]  WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
HAE1    TAVYYCAR[GS HYFGHWHFAV]  WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
Hu-901  TAVYYCAR [GS HFSGSNYDYFDY] WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS

E25       VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
E26       VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
HAE1      VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
Hu-901    VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

E25       VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
E26       VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
HAE1      VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
Hu-901    VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE

E25       SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 41
E26       SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 42
HAE1      SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 43
Hu-901    SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 44
```

FIG. 17B

Sequence Alignment of Variable Light Domains

```
                  ├──────FR1──────┤      CDR1      ├──
                      10        20        30          40
2H7               QIVLSQSPAILSASPGEKVTMTC [RASSSVS-YMH] WYQQKP
                  * ***   **    * **
hu2H7.v16         DIQMTQSPSSLSASVGDRVTITC [RASSSVS-YMH] WYQQKP
                                  * * * **
hum kI            DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                  ─FR2─┤   CDR2   ├──────────FR3──────
                     50          60        70        80
2H7               GSSPKPWIY [APSNLAS] GVPARFSGSGSGTSYSLTISRVEA
                   **  *             *       ***   ****
hu2H7.v16         GKAPKPLIY [APSNLAS] GVPSRFSGSGSGTDFTLTISSLQP
                      *     * * *
hum kI            GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                  ──────┤   CDR3   ├─FR4─┤
                     90          100
2H7               EDAATYYC [QQWSFNPPT] FGAGTKLELKR
                    *                   *  * *
hu2H7.v16         EDFATYYC [QQWSFNPPT] FGQGTKVEIKR
                           * **** *
hum kI            EDFATYYC [QQYNSLPWT] FGQGTKVEIKR
```

FIG. 18A

Sequence Alignment of Variable Heavy Domains

```
                  ├──────FR1──────┤      CDR1      ├──
                      10        20        30          40
2H7               QAYLQQSGAELVRPGASVKMSCKAS [GYTFTSYNMH] WVKQT
                  *** **  **  *  * *** *                 * *
hu2H7.v16         EVQLVESGGGLVQPGGSLRLSCAAS [GYTFTSYNMH] WVRQA
                                            * *  * *
hum III           EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                  ─FR2─┤      CDR2      ├─────FR3──────
                     50 a        60          70        80
2H7               PRQGLEWIG [AIYPGNGDTSYNQKFKG] KATLTVDKSSSTAYM
                   **   *                       ** **   ** * *
hu2H7.v16         PGKGLEWVG [AIYPGNGDTSYNQKFKG] RFTISVDKSKNTLYL
                          *  *  **** * * ****       * *
hum III           PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                  ──────────────┤      CDR3      ├─FR4─┤
                    abc     90          100abcde      110
2H7               QLSSLTSEDSAVYFCAR [VVYYSNSYWYFDV] WGTGTTVTVSS
                   **  **  *   *                     *
hu2H7.v16         QMNSLRAEDTAVYYCAR [VVYYSNSYWYFDV] WGQGTLVTVSS
                                    ******* *** *
hum III           QMNSLRAEDTAVYYCAR [GRVGYSLY---DY] WGQGTLVTVSS
```

FIG. 18B

Anti-VEGF Antibody Variable Domain Sequences

anti-VEGF variable light sequence:

DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKPDGTVKVLIYFTSSLHSGVPSRFSGS
GSGTDYSLTISNLEPEDIATYYCQQYSTVPWTFGGGTKLEIK
(SEQ ID NO: 31)

anti-VEGF variable heavy sequence of:

EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGEPTYAADFK
RRFTFSLETSASTAYLQISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGAGTTVTVSS
(SEQ ID NO: 32)

anti-VEGF antibody variable light sequence:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK
(SEQ ID NO: 33)

anti-VEGF antibody variable heavy sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFK
RRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS
(SEQ ID NO: 34)

anti-VEGF antibody variable light sequence:

DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK
(SEQ ID NO: 35)

anti-VEGF antibody variable heavy sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFK
RRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSS
(SEQ ID NO: 36)

FIG. 19

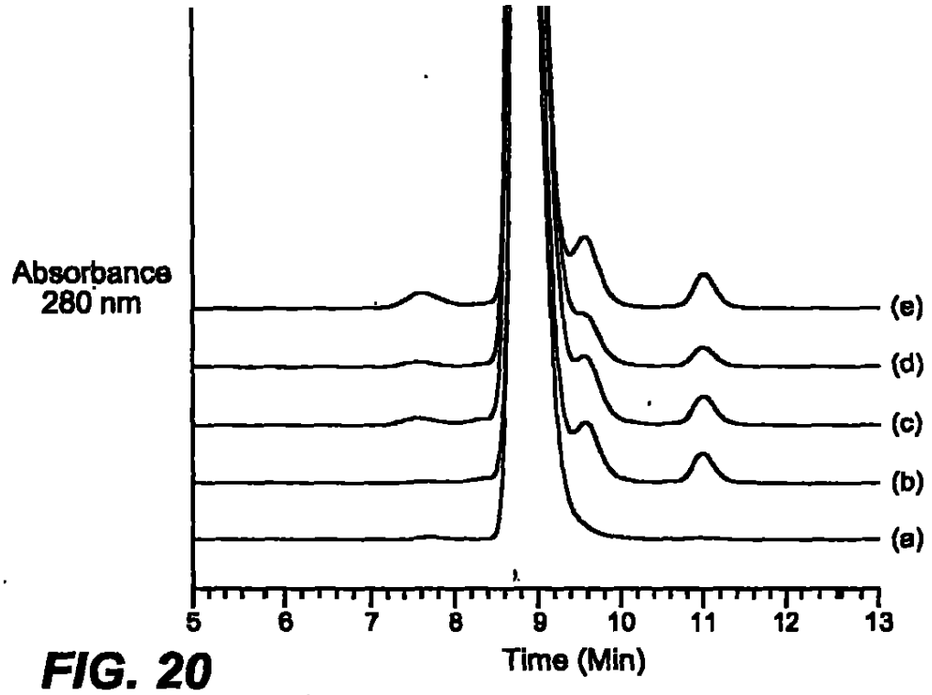

Absorbance
280 nm
(e)
(d)
(c)
(b)
(a)
5
6
7
8
9
10
11
12
13
Time (Min)

FIG. 20

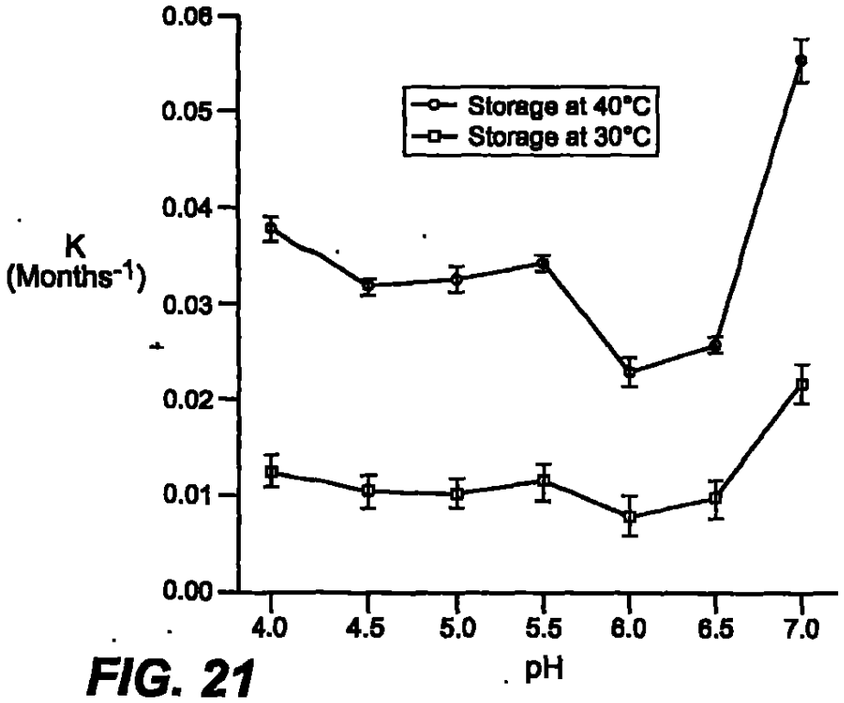

Storage at 40°C
Storage at 30°C
K
(Months-1)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH

FIG. 21

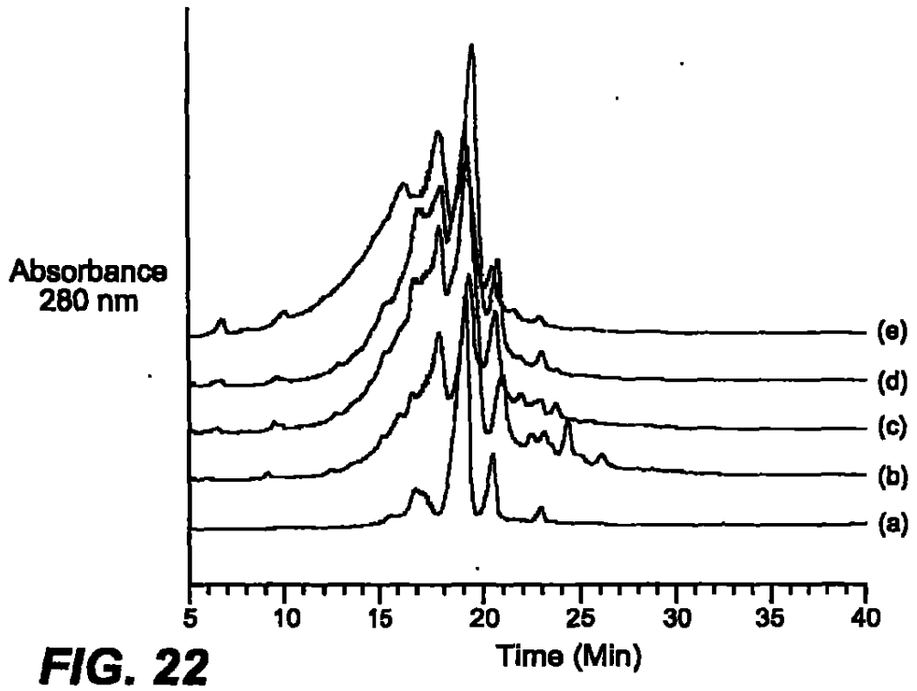

Absorbance
280 nm
(e)
(d)
(c)
(b)
(a)
5
10
15
20
25
30
35
40
Time (Min)

FIG. 22

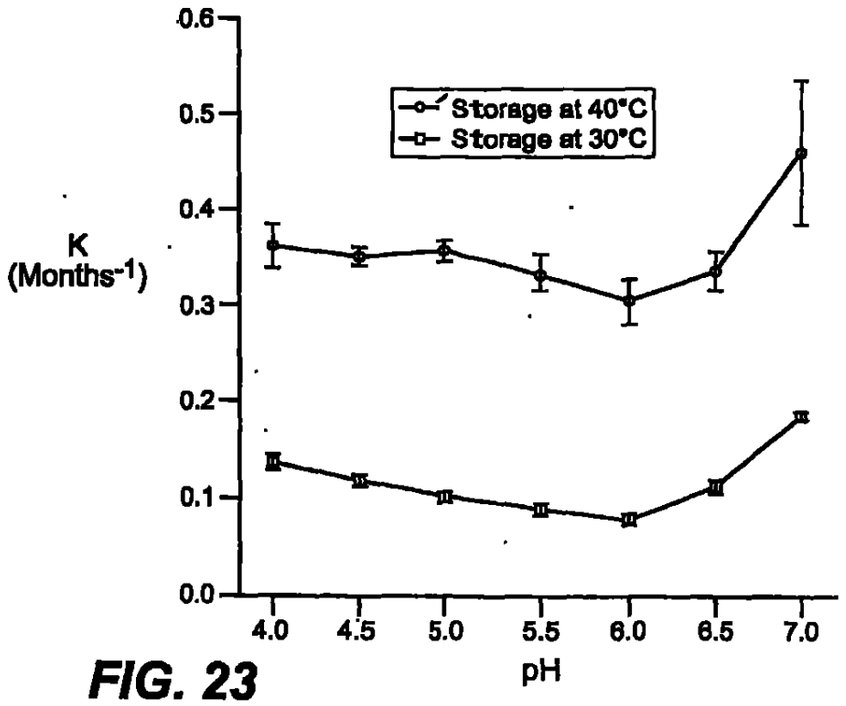

Storage at 40°C
Storage at 30°C
K
(Months-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH

FIG. 23

```
   1 TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

   1 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
   1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

 181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
  31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu

 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
  61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

 361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCMCAG
  91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln

 451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

 541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

 631 TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

 721 TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

 811 TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
 241 SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

 901 GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp

 991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG. 24

```
   1 CCCACGCGTC CGCATAAATC AGCACGCGGC CGGAGAACCC CGCAATCTCT GCGCCCACAA AATACACCGA CGATGCCCGA TCTACTTTAA GGGCTGAAAC
     GGGTGCGCAG GCGTATTTAG TCGTGCGCCG GCCTCTTGGG GCGTTAGAGA CGCGGGTGTT TTATGTGGCT GCTACGGGCT AGATGAAATT CCCGACTTTG

 101 CCACGGGCCT GAGAGACTAT AAGAGCGTTC CCTACCGCCA TGGAACAACG GGGACAGAAC GCCCCGGCCG CTTCGGGGGC CCGGAAAAGG CACGGCCCAG
     GGTGCCCGGA CTCTCTGATA TTCTCGCAAG GGATGGCGGT ACCTTGTTGC CCCTGTCTTG CGGGGCCGGC GAAGCCCCCG GGCCTTTTCC GTGCCGGGTC
   1                                    M etGluGlnAr gGlyGlnAsn AlaProAlaA laSerGlyAl aArgLysArg HisGlyProGly

 201 GACCCAGGGA GGCGCGGGGA GCCAGGCCTG GGCTCCGGGT CCCCAAGACC CTTGTGCTCG TTGTCGCCGC GGTCCTGCTG TTGGTCTCAG CTGAGTCTGC
     CTGGGTCCCT CCGCGCCCCT CGGTCCGGAC CCGAGGCCCA GGGGTTCTGG GAACACGAGC AACAGCGGCG CCAGGACGAC AACCAGAGTC GACTCAGACG
  22   ProArgGl uAlaArgGly AlaArgProG lyLeuArgVa lProLysThr LeuValLeuV alValAlaAl aValLeuLeu LeuValSerA laGluSerAla

 301 TCTGATCACC CAACAAGACC TAGCTCCCCA GCAGAGAGCG GCCCCACAAC AAAAGAGGTC CAGCCCCTCA GAGGGATTGT GTCCACCTGG ACACCATATC
     AGACTAGTGG GTTGTTCTGG ATCGAGGGGT CGTCTCTCGC CGGGGTGTTG TTTTCTCCAG GTCGGGGAGT CTCCCTAACA CAGGTGGACC TGTGGTATAG
  55  LeuIleThr GlnGlnAspL euAlaProGl nGlnArgAla AlaProGlnG lnLysArgSe rSerProSer GluGlyLeuC ysProProGl yHisHisIle

 401 TCAGAAGACG GTAGAGATTG CATCTCCTGC AAATATGGAC AGGACTATAG CACTCACTGG AATGACCTCC TTTTCTGCTT GCGCTGCACC AGGTGTGATT
     AGTCTTCTGC CATCTCTAAC GTAGAGGACG TTTATACCTG TCCTGATATC GTGAGTGACC TTACTGGAGG AAAAGACGAA CGCGACGTGG TCCACACTAA
  88 SerGluAspG lyArgAspCy sIleSerCys LysTyrGlyG lnAspTyrSe rThrHisTrp AsnAspLeuL euPheCysLe uArgCysThr ArgCysAspSer

 501 CAGGTGAAGT GGAGCTAAGT CCCTGCACCA CGACCAGAAA CACAGTGTGT CAGTGCGAAG AAGGCACCTT CCGGGAAGAA GATTCTCCTG AGATGTGCCG
     GTCCACTTCA CCTCGATTCA GGGACGTGGT GCTGGTCTTT GTGTCACACA GTCACGCTTC TTCCGTGGAA GGCCCTTCTT CTAAGAGGAC TCTACACGGC
 122   GlyGluVa lGluLeuSer ProCysThrT hrThrArgAs nThrValCys GlnCysGluG luGlyThrPh eArgGluGlu AspSerProG luMetCysArg

 601 GAAGTGCCGC ACAGGGTGTC CCAGAGGGAT GGTCAAGGTC GGTGATTGTA CACCCTGGAG TGACATCGAA TGTGTCCACA AAGAATCAGG CATCATCATA
     CTTCACGGCG TGTCCCACAG GGTCTCCCTA CCAGTTCCAG CCACTAACAT GTGGGACCTC ACTGTAGCTT ACACAGGTGT TTCTTAGTCC GTAGTAGTAT
 155  LysCysArg ThrGlyCysP roArgGlyMe tValLysVal GlyAspCysT hrProTrpSe rAspIleGlu CysValHisL ysGluSerGl yIleIleIle

 701 GGAGTCACAG TTGCAGCCGT AGTCTTGATT GTGGCTGTGT TTGTTTGCAA GTCTTTACTG TGGAAGAAAG TCCTTCCTTA CCTGAAAGGC ATCTGCTCAG
     CCTCAGTGTC AACGTCGGCA TCAGAACTAA CACCGACACA AACAAACGTT CAGAAATGAC ACCTTCTTTC AGGAAGGAAT GGACTTTCCG TAGACGAGTC
 188 GlyValThrV alAlaAlaVa lValLeuIle ValAlaValP heValCysLy sSerLeuLeu TrpLysLysV alLeuProTy rLeuLysGly IleCysSerGly

 801 GTGGTGGTGG GGACCCTGAG CGTGTGGACA GAAGCTCACA ACGACCTGGG GCTGAGGACA ATGTCCTCAA TGAGATCGTG AGTATCTTGC AGCCCACCCA
     CACCACCACC CCTGGGACTC GCACACCTGT CTTCGAGTGT TGCTGGACCC CGACTCCTGT TACAGGAGTT ACTCTAGCAC TCATAGAACG TCGGGTGGGT
 222   GlyGlyGl yAspProGlu ArgValAspA rgSerSerGl nArgProGly AlaGluAspA snValLeuAs nGluIleVal SerIleLeuG lnProThrGln
```

FIG. 25A

```
 901 GGTCCCTGAG CAGGAAATGG AAGTCCAGGA GCCAGCAGAG CCAACAGGTG TCAACATGTT GTCCCCCGGG GAGTCAGAGC ATCTGCTGGA ACCGGCAGAA
     CCAGGGACTC GTCCTTTACC TTCAGGTCCT CGGTCGTCTC GGTTGTCCAC AGTTGTACAA CAGGGGGCCC CTCAGTCTCG TAGACGACCT TGGCCGTCTT
 255  ValProGlu GlnGluMetG luValGlnGl uProAlaGlu ProThrGlyV alAsnMetLe uSerProGly GluSerGluH isLeuLeuGl uProAlaGlu

1001 GCTGAAAGGT CTCAGAGGAG GAGGCTGCTG GTTCCAGCAA ATGAAGGTGA TCCCACTGAG ACTCTGAGAC AGTGCTTCGA TGACTTTGCA GACTTGGTGC
     CGACTTTCCA GAGTCTCCTC CTCCGACGAC CAAGGTCGTT TACTTCCACT AGGGTGACTC TGAGACTCTG TCACGAAGCT ACTGAAACGT CTGAACCACG
 288 AlaGluArgS erGlnArgAr gArgLeuLeu ValProAlaA snGluGlyAs pProThrGlu ThrLeuArgG lnCysPheAs pAspPheAla AspLeuValPro

1101 CCTTTGACTC CTGGGAGCCG CTCATGAGGA AGTTGGGCCT CATGGACAAT GAGATAAAGG TGGCTAAAGC TGAGGCAGCG GGCCACAGGG ACACCTTGTA
     GGAAACTGAG GACCCTCGGC GAGTACTCCT TCAACCCGGA GTACCTGTTA CTCTATTTCC ACCGATTTCG ACTCCGTCGC CCGGTGTCCC TGTGGAACAT
 322   PheAspSe rTrpGluPro, LeuMetArgL ysLeuGlyLe uMetAspAsn GluIleLysV alAlaLysAl aGluAlaAla GlyHisArgA spThrLeuTyr

1201 CACGATGCTG ATAAAGTGGG TCAACAAAAC CGGGCGAGAT GCCTCTGTCC ACACCCTGCT GGATGCCTTG GAGACGCTGG GAGAGAGACT TGCCAAGCAG
     GTGCTACGAC TATTTCACCC AGTTGTTTTG GCCCGCTCTA CGGAGACAGG TGTGGGACGA CCTACGGAAC CTCTGCGACC CTCTCTCTGA ACGGTTCGTC
 355  ThrMetLeu IleLysTrpV alAsnLysTh rGlyArgAsp AlaSerValH isThrLeuLe uAspAlaLeu GluThrLeuG lyGluArgLe uAlaLysGln

1301 AAGATTGAGG ACCACTTGTT GAGCTCTGGA AAGTTCATGT ATCTAGAAGG TAATGCAGAC TCTGCCWTGT CCTAAGTGTG ATTCTCTTCA GGAAGTGAGA
     TTCTAACTCC TGGTGAACAA CTCGAGACCT TTCAAGTACA TAGATCTTCC ATTACGTCTG AGACGGAACA GGATTCACAC TAAGAGAAGT CCTTCACTCT
 388 LysIleGluA spHisLeuLe uSerSerGly LysPheMetT yrLeuGluGl yAsnAlaAsp SerAlaXaaS erOC*

1401 CCTTCCCTGG TTTACCTTTT TTCTGGAAAA AGCCCAACTG GACTCCAGTC AGTAGGAAAG TGCCACAATT GTCACATGAC CGGTACTGGA AGAAACTCTC
     GGAAGGGACC AAATGGAAAA AAGACCTTTT TCGGGTTGAC CTGAGGTCAG TCATCCTTTC ACGGTGTTAA CAGTGTACTG GCCATGACCT TCTTTGAGAG

1501 CCATCCAACA TCACCCAGTG GATGGAACAT CCTGTAACTT TTCACTGCAC TTGGCATTAT TTTTATAAGC TGAATGTGAT AATAAGGACA CTATGGAAAT
     GGTAGGTTGT AGTGGGTCAC CTACCTTGTA GGACATTGAA AAGTGACGTG AACCGTAATA AAAATATTCG ACTTACACTA TTATTCCTGT GATACCTTTA

1601 GTCTGGATCA TTCCGTTTGT GCGTACTTTG AGATTTGGTT TGGGATGTCA TTGTTTTCAC AGCACTTTTT TATCCTAATG TAAATGCTTT ATTTATTTAT
     CAGACCTAGT AAGGCAAACA CGCATGAAAC TCTAAACCAA ACCCTACAGT AACAAAAGTG TCGTGAAAAA ATAGGATTAC ATTTACGAAA TAAATAAATA

1701 TTGGGCTACA TTGTAAGATC CATCTACAAA AAAAAAAAAA AAAAAAAAAG GGCGGCCGCG ACTCTAGAGT CGACCTGCAG AAGCTTGGCC GCCATGGCC
     AACCCGATGT AACATTCTAG GTAGATGTTT TTTTTTTTTT TTTTTTTTTC CCGCCGGCGC TGAGATCTCA GCTGGACGTC TTCGAACCGG CGGTACCGG
```

FIG. 25B

```
ATG GAA CAA CGG GGA CAG AAC GCC CCG GCC GCT TCG GGG GCC CGG AAA     48
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
  1               5                  10                  15

AGG CAC GGC CCA GGA CCC AGG GAG GCG CGG GGA GCC AGG CCT GGG CCC     96
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
            20                  25                  30

CGG GTC CCC AAG ACC CTT GTG CTC GTT GTC GCC GCG GTC CTG CTG TTG    144
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
        35                  40                  45

GTC TCA GCT GAG TCT GCT CTG ATC ACC CAA CAA GAC CTA GCT CCC CAG    192
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
    50                  55                  60

CAG AGA GCG GCC CCA CAA CAA AAG AGG TCC AGC CCC TCA GAG GGA TTG    240
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
65                  70                  75                  80

TGT CCA CCT GGA CAC CAT ATC TCA GAA GAC GGT AGA GAT TGC ATC TCC    288
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                85                  90                  95

TGC AAA TAT GGA CAG GAC TAT AGC ACT CAC TGG AAT GAC CTC CTT TTC    336
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu Leu Phe
            100                 105                 110

TGC TTG CGC TGC ACC AGG TGT GAT TCA GGT GAA GTG GAG CTA AGT CCG    384
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125

TGC ACC ACG ACC AGA AAC ACA GTG TGT CAG TGC GAA GAA GGC ACC TTC    432
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140

CGG GAA GAA GAT TCT CCT GAG ATG TGC CGG AAG TGC CGC ACA GGG TGT    480
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160

CCC AGA GGG ATG GTC AAG GTC GGT GAT TGT ACA CCC TGG AGT GAC ATC    528
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175

GAA TGT GTC CAC AAA GAA TCA GGT ACA AAG CAC AGT GGG GAA GCC CCA    576
Glu Cys Val His Lys Glu Ser Gly Thr Lys His Ser Gly Glu Ala Pro
            180                 185                 190

GCT GTG GAG GAG ACG GTG ACC TCC AGC CCA GGG ACT CCT GCC TCT CCC    624
Ala Val Glu Glu Thr Val Thr Ser Ser Pro Gly Thr Pro Ala Ser Pro
        195                 200                 205

TGT TCT CTC TCA GGC ATC ATC ATA GGA GTC ACA GTT GCA GCC GTA GTC    672
Cys Ser Leu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
    210                 215                 220
```

FIG. 26A

```
TTG ATT GTG GCT GTG TTT GTT TGC AAG TCT TTA CTG TGG AAG AAA GTC   720
Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys Val
225                 230                 235                 240

CTT CCT TAC CTG AAA GGC ATC TGC TCA GGT GGT GGT GGG GAC CCT GAG   768
Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp Pro Glu
                245                 250                 255

CGT GTG GAC AGA AGC TCA CAA CGA CCT GGG GCT GAG GAC AAT GTC CTC   816
Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp Asn Val Leu
            260                 265                 270

AAT GAG ATC GTG AGT ATC TTG CAG CCC ACC CAG GTC CCT GAG CAG GAA   864
Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro Glu Gln Glu
        275                 280                 285

ATG GAA GTC CAG GAG CCA GCA GAG CCA ACA GGT GTC AAC ATG TTG TCC   912
Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn Met Leu Ser
    290                 295                 300

CCC GGG GAG TCA GAG CAT CTG CTG GAA CCG GCA GAA GCT GAA AGG TCT   960
Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala Glu Arg Ser
305                 310                 315                 320

CAG AGG AGG AGG CTG CTG GTT CCA GCA AAT GAA GGT GAT CCC ACT GAG  1008
Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp Pro Thr Glu
                325                 330                 335

ACT CTG AGA CAG TGC TTC GAT GAC TTT GCA GAC TTG GTG CCC TTT GAC  1056
Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val Pro Phe Asp
            340                 345                 350

TCC TGG GAG CCG CTC ATG AGG AAG TTG GGC CTC ATG GAC AAT GAG ATA  1104
Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp Asn Glu Ile
        355                 360                 365

AAG GTG GCT AAA GCT GAG GCA GCG GGC CAC AGG GAC ACC TTG TAC ACG  1152
Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr Leu Tyr Thr
    370                 375                 380

ATG CTG ATA AAG TGG GTC AAC AAA ACC GGG CGA GAT GCC TCT GTC CAC  1200
Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala Ser Val His
385                 390                 395                 400

ACC CTG CTG GAT GCC TTG GAG ACG CTG GGA GAG AGA CTT GCC AAG CAG  1248
Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln
                405                 410                 415

AAG ATT GAG GAC CAC TTG TTG AGC TCT GGA AAG TTC ATG TAT CTA GAA  1296
Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu
            420                 425                 430

GGT AAT GCA GAC TCT GCC ATG TCC TAA                             1323
Gly Asn Ala Asp Ser Ala Met Ser  *
        435                 440
```

FIG. 26B

EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSGINWQGGSTGY
ADSVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

<451 residues; 0 stop; molecular weight: 49167.50

FIG. 27

SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIYGANNRPSGIPDRF
SGSSSGNTASLTITGAQAEDEADYYCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS
LTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

<213 residues; 0 stop; molecular weight: 22400.70

FIG. 28

Alignment, 16ES and Apomab 7.3 Heavy Chains

```
16E2           1 EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYGMSW
                                         *
Apomab7.3      1 EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSW

16E2          37 VRQAPGKGLEWVSGINWNGGSTGYADSVKGRVTISRDNAKNSLYLQMNSL
                                 *
Apomab7.3     37 VRQAPGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLYLQMNSL

16E2          87 RAEDTAVYYCAKILGAGRGWYFDLWGKGTTVTVSSASTKGPSVFPLAPSS
                                          *
Apomab7.3     87 RAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVSSASTKGPSVFPLAPSS

16E2         137 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
Apomab7.3    137 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL

16E2         187 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
Apomab7.3    187 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP

16E2         237 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
Apomab7.3    237 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV

16E2         287 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
Apomab7.3    287 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI

16E2         337 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
Apomab7.3    337 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE

16E2         387 SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
Apomab7.3    387 SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

16E2.huIgG1  437 HNHYTQKSLSLSPGK
Apomab7.3    437 HNHYTQKSLSLSPGK
```

FIG. 29

Alignment, 16E2 and Apomab 7.3 Light Chains

```
16E2          1 SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKN
                                      *                   *
Apomab7.3     1 SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIYGAN

16E2         51 NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGG
                                                  *
Apomab7.3    51 NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSADSSGNHVVFGG

16E2        101 GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
Apomab7.3   101 GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK

16E2        151 ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEG
Apomab7.3   151 ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEG

16E2        201 STVEKTVAPTECS
Apomab7.3   201 STVEKTVAPTECS
```

FIG. 30

Apomab Variable Heavy Domain Sequence

CDR H1
EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMWVRQAPGKGLEWV

CDR H2
SGINWQGGSTGYADSVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAK

CDR H3
ILGRGRGWYFDYWGKGTTVTVSSASTKGP

FIG. 31A

Apomab Variable Light Domain Sequence

CDR L1
SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIY

CDR L2 CDRL3
GANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSADSSGNEVV

FGGGTKLTVLG

FIG. 31B

Light Chain Alignment

```
                1                                32
hu2H7.v16       DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP
                ********************************.*********************
hu2H7.v511      DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP

               52
hu2H7.v16       SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG
                ********************************************.*********
hu2H7.v511      SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG

              102
hu2H7.v16       TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
                ************************************************************
hu2H7.v511      TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD

              152
hu2H7.v16       NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
                ************************************************************
hu2H7.v511      NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL

              202         214
hu2H7.v16       SSPVTKSFNRGEC
                *************
hu2H7.v511      SSPVTKSFNRGEC
```

FIG. 32

Heavy Chain Alignment

```
                1
hu2H7.v16       EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
                ************************************
hu2H7.v511      EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW

               37              52a                                82abc
hu2H7.v16       VRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSL
                ********************* ********************************
hu2H7.v511      VRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL

               83              100abcde          113
hu2H7.v16       RAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS
                ******************  ******************
hu2H7.v511      RAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS

              118
hu2H7.v16       ASTKGPSVFPLAPS
                **************
hu2H7.v511      ASTKGPSVFPLAPS

              132
hu2H7.v16       SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
                ******************************************************
hu2H7.v511      SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS

              182
hu2H7.v16       LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
                ******************************************************
hu2H7.v511      LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA

              232
hu2H7.v16       PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
                ******************************************************
hu2H7.v511      PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG

              282
hu2H7.v16       VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
                ****************.****************************** *****
hu2H7.v511      VEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAP

              332
hu2H7.v16       IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
                *  **************************************************
hu2H7.v511      IAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW

              382
hu2H7.v16       ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
                ******************************************************
hu2H7.v511      ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

              432             447
hu2H7.v16       LHNHYTQKSLSLSPGK
                ****************
hu2H7.v511      LHNHYTQKSLSLSPGK
```

FIG. 33

103.411

H2
121

Sequence Listing

<110> GENENTECH, INC.
ANDYA, JAMES
GWEE, SHIANG C.
LIU, JUN
SHEN,YE

<120> ANTIBODY FORMULATIONS

<130> P2104R1 PCT

<140> PCT/US2005/037471
<141> 2005-10-19

<150> US 60/620,413
<151> 2004-10-20

<160> 74

<210> 1
<211> 107
<212> PRT
<213> Mus musculus

<400> 1
Asp Thr Val Met Thr Gln Ser His Lys Ile Met Ser Thr Ser Val
1 5 10 15

Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

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

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp
50 55 60

Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile
65 70 75

Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 100 105

Ile Lys

<210> 2
<211> 119
<212> PRT
<213> Mus musculus

<400> 2
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
1 5 10 15

Thr Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Thr Phe Thr
20 25 30

Asp Tyr Thr Met Asp Trp Val Lys Gln Ser His Gly Lys Ser Leu
35 40 45

Glu Trp Ile Gly Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60

Asn Gln Arg Phe Lys Gly Lys Ala Ser Leu Thr Val Asp Arg Ser
65 70 75

Ser Arg Ile Val Tyr Met Glu Leu Arg Ser Leu Thr Phe Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
95 100 105

Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110 115

<210> 3
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> sequence is synthesized

<400> 3
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 4
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> sequence is synthesized

<400> 4
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
95 100 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 5
<211> 107
<212> PRT
<213> Artificial Sequence

<220>

~~120~~
123

<223> sequence is synthesized

<400> 5
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30

Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 6
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> sequence is synthesized

<400> 6
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
95 100 105

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 7
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<220>
<221> Xaa
<222> 10
<223> Xaa is preferrably D or S

<400> 7
Gly Phe Thr Phe Thr Asp Tyr Thr Met Xaa
5 10

<210> 8
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 8
Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr Asn Gln Arg Phe
1 5 10 15

Lys Gly

<210> 9
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 9
Asn Leu Gly Pro Ser Phe Tyr Phe Asp Tyr
5 10

<210> 10
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 10
Lys Ala Ser Gln Asp Val Ser Ile Gly Val Ala
5 10

<210> 11
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<220>
<221> Xaa
<222> 5
<223> Xaa is preferably R or L.

<220>
<221> Xaa
<222> 6
<223> Xaa is preferably Y or E.

<220>
<221> Xaa
<222> 7
<223> Xaa is preferably T or S.

<400> 11
Ser Ala Ser Tyr Xaa Xaa Xaa
5

<210> 12
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 12

Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr
5

<210> 13
<211> 214
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 13
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 14
<211> 449
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 14
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr

```
                50                  55                  60

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

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105

Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
               110                 115                 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
               125                 130                 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
               140                 145                 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
               155                 160                 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
               170                 175                 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
               185                 190                 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
               200                 205                 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
               215                 220                 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
               230                 235                 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
               260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
               275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
               290                 295                 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
               305                 310                 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
               320                 325                 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
               335                 340                 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
               350                 355                 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
               365                 370                 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
               380                 385                 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
               395                 400                 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
               410                 415                 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
               425                 430                 435
```

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445

<210> 15
<211> 214
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 15
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 16
<211> 448
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

```
Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
                50                  55                  60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
                65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
                95                 100                 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
               110                 115                 120

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
               125                 130                 135

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
               140                 145                 150

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
               155                 160                 165

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
               170                 175                 180

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
               185                 190                 195

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
               200                 205                 210

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
               215                 220                 225

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
               230                 235                 240

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

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
               260                 265                 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
               275                 280                 285

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

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
               305                 310                 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
               320                 325                 330

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
               335                 340                 345

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
               350                 355                 360

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
               365                 370                 375

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
               380                 385                 390

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
               395                 400                 405

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
               410                 415                 420

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
```

425 430 435

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445

<210> 17
<211> 233
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 17
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
20 25 30

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

Gln Asp Val Ser Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly
50 55 60

Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr
65 70 75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
80 85 90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
95 100 105

Tyr Cys Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly
110 115 120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
125 130 135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
140 145 150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
155 160 165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
170 175 180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
185 190 195

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
200 205 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
215 220 225

Lys Ser Phe Asn Arg Gly Glu Cys
230

<210> 18
<211> 467
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 18
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
20 25 30

```
Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
                35                  40                  45

Phe Thr Phe Thr Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro
                50                  55                  60

Gly Lys Gly Leu Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly
                65                  70                  75

Gly Ser Ile Tyr Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser
                80                  85                  90

Val Asp Arg Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
                95                  100                 105

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly
                110                 115                 120

Pro Ser Phe Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
                125                 130                 135

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
                140                 145                 150

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
                155                 160                 165

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
                170                 175                 180

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                185                 190                 195

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
                200                 205                 210

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
                215                 220                 225

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
                230                 235                 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
                245                 250                 255

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
                260                 265                 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                275                 280                 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                290                 295                 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
                305                 310                 315

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
                320                 325                 330

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
                335                 340                 345

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
                350                 355                 360

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
                365                 370                 375

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                380                 385                 390

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
                395                 400                 405
```

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
410 415 420

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
425 430 435

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
440 445 450

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
455 460 465

Pro Gly

<210> 19
<211> 195
<212> PRT
<213> Homo sapiens

<400> 19
Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala
1 5 10 15

Ser Pro Glu Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly
20 25 30

Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr Leu Pro Thr
35 40 45

Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val Gln Gly
50 55 60

Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln
65 70 75

Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
80 85 90

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr
95 100 105

Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu
110 115 120

Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg
125 130 135

Asn Pro Gln Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile
140 145 150

Phe His Lys Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn
155 160 165

Arg Ser Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys Gly Ser
170 175 180

Arg Cys Trp Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg
185 190 195

<210> 20
<211> 124
<212> PRT
<213> Homo sapiens

<400> 20
Thr Val Cys Ala Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro
1 5 10 15

Thr Asp Cys Cys His Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro
20 25 30

Lys His Ser Asp Cys Leu Ala Cys Leu His Phe Asn His Ser Gly
35 40 45

Ile Cys Glu Leu His Cys Pro Ala Leu Val Thr Tyr Asn Thr Asp

50 55 60

Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg Tyr Thr Phe Gly
65 70 75

Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu Ser Thr Asp
80 85 90

Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln Glu Val
95 100 105

Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys Pro
110 115 120

Cys Ala Arg Val

<210> 21
<211> 169
<212> PRT
<213> Homo sapiens

<400> 21
Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu Val Arg Ala Val
1 5 10 15

Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys Lys Ile Phe
20 25 30

Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp Pro Ala
35 40 45

Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe Glu
50 55 60

Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
65 70 75

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile
80 85 90

Arg Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln
95 100 105

Gly Leu Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu
110 115 120

Gly Ser Gly Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe
125 130 135

Val His Thr Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln
140 145 150

Ala Leu Leu His Thr Ala Asn Arg Pro Glu Asp Glu Cys Val Gly
155 160 165

Glu Gly Leu Ala

<210> 22
<211> 142
<212> PRT
<213> Homo sapiens

<400> 22
Cys His Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro
1 5 10 15

Thr Gln Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys
20 25 30

Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val
35 40 45

Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln
50 55 60

Asn Gly Ser Val Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val
65 70 75

Ala Cys Ala His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg Cys
80 85 90

Pro Ser Gly Val Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys
95 100 105

Phe Pro Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn Cys
110 115 120

Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly Cys Pro Ala Glu
125 130 135

Gln Arg Ala Ser Pro Leu Thr
140

<210> 23
<211> 217
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 23
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
1 5 10 15

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln
20 25 30

Asp Val Ser Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys
35 40 45

Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly
50 55 60

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
65 70 75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
80 85 90

Cys Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr
95 100 105

Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile
110 115 120

Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
125 130 135

Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
140 145 150

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
155 160 165

Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
170 175 180

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
185 190 195

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
200 205 210

Ser Phe Asn Arg Gly Glu Cys
215

<210> 24
<211> 449
<212> PRT

<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 24

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

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
             35                  40                  45

Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
             50                  55                  60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
             65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
             80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
             95                 100                 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
            110                 115                 120

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
            125                 130                 135

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
            140                 145                 150

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
            155                 160                 165

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
            170                 175                 180

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            185                 190                 195

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
            200                 205                 210

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
            215                 220                 225

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
            230                 235                 240

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

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
            275                 280                 285

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

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            305                 310                 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            320                 325                 330

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
            335                 340                 345

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
```

```
                350                 355                 360

 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                365                 370                 375

 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
                380                 385                 390

 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
                395                 400                 405

 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                410                 415                 420

 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
                425                 430                 435

 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                440                 445

<210> 25
<211> 107
<212> PRT
<213> Mus musculus

<400> 25
 Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro
   1               5                  10                  15

 Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser
                20                  25                  30

 Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro
                35                  40                  45

 Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
                50                  55                  60

 Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser
                65                  70                  75

 Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
                80                  85                  90

 Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
                95                 100                 105

 Lys Arg


<210> 26
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 26
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
   1               5                  10                  15

 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
                20                  25                  30

 Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
                35                  40                  45

 Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
                50                  55                  60

 Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                65                  70                  75

 Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
                80                  85                  90
```

Ser Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105

Lys Arg

<210> 27
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> sequence is synthesized

<400> 27
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30

Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg

<210> 28
<211> 122
<212> PRT
<213> Mus musculus

<400> 28
Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly
1 5 10 15

Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu
35 40 45

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr Val
110 115 120

Ser Ser

<210> 29
<211> 122
<212> PRT

<213> Artificial sequence

<220>
<223> sequence is synthesized

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser

<210> 30
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> sequence is synthesized

<400> 30
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
95 100 105

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 31
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 31
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu

```
  1               5                  10                  15

Gly Asp Arg Val Ile Ile Ser Cys Ser Ala Ser Gln Asp Ile Ser
                20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
                35                  40                  45

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                65                  70                  75

Ser Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
                95                 100                 105

Ile Lys
```

<210> 32
<211> 123
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 32

```
Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Gln Pro Gly
  1               5                  10                  15

Glu Thr Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
                20                  25                  30

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

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
                50                  55                  60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Glu Thr Ser
                65                  70                  75

Ala Ser Thr Ala Tyr Leu Gln Ile Ser Asn Leu Lys Asn Asp Asp
                80                  85                  90

Thr Ala Thr Tyr Phe Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
                95                 100                 105

Ser His Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr
               110                 115                 120

Val Ser Ser
```

<210> 33
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 33

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser
                20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45
```

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 34
<211> 123
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 34
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
50 55 60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
65 70 75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
95 100 105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser

<210> 35
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 35
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 36
<211> 123
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 36
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
50 55 60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
65 70 75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr
95 100 105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser

<210> 37
<211> 218
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 37
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp
20 25 30

Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly
35 40 45

Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser
50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
80 85 90

Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly
95 100 105

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe

110 115 120

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
125 130 135

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
140 145 150

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
155 160 165

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
170 175 180

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
185 190 195

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
200 205 210

Lys Ser Phe Asn Arg Gly Glu Cys
215

<210> 38
<211> 218
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 38
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Pro Val Asp
20 25 30

Gly Glu Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
35 40 45

Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser
50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
80 85 90

Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly
95 100 105

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
110 115 120

Ile Phe Pro Pro Ser Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
125 130 135

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
140 145 150

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
155 160 165

Ser Phe Asn Arg Gly Glu Cys Asp Glu Gln Leu Lys Ser Gly Thr
170 175 180

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
185 190 195

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
200 205 210

Gln Glu Ser Val Thr Glu Gln Asp
215

<210> 39
<211> 218
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 39

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	
1				5				10						15	
Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Lys	Pro	Val	Asp	
				20				25						30	
Gly	Glu	Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	
				35				40						45	
Lys	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Tyr	Leu	Glu	Ser	
				50				55						60	
Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
				65				70						75	
Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	
				80				85						90	
Tyr	Cys	Gln	Gln	Ser	His	Glu	Asp	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	
				95				100						105	
Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	
				110				115						120	
Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	
				125				130						135	
Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	
				140				145						150	
Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	
				155				160						165	
Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
				170				175						180	
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
				185				190						195	
Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	
				200				205						210	
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys								
				215											

<210> 40
<211> 214
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 40

Asp	Ile	Leu	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	
1				5				10						15	
Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Gly	
				20				25						30	
Thr	Asn	Ile	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	
				35				40						45	
Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Glu	Ser	Ile	Ser	Gly	Ile	Pro	Ser	
				50				55						60	

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Arg Leu Glu Pro Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln
80 85 90

Ser Asp Ser Trp Pro Thr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 41
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 41
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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

Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr
50 55 60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
95 100 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
110 115 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser

```
                170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
                200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
                215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
                290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
                350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                410                 415                 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                425                 430                 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                440                 445                 450

Lys
```

<210> 42
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 42

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

 Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
                20                  25                  30

 Ser Gly Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly
                35                  40                  45
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```
Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr
                50                  55                  60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
                95                 100                 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
               110                 115                 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
               125                 130                 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
               140                 145                 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
               155                 160                 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
               170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
               185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
               200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
               215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
               230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
               245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
               260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
               275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
               290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
               305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
               320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
               335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
               350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
               365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
               380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
               395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
               410                 415                 420
```

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 43
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 43
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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

Leu Glu Trp Val Ala Ser Ile Lys Tyr Ser Gly Glu Thr Lys Tyr
50 55 60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
95 100 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
110 115 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
170 175 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185 190 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200 205 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215 220 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr

290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
320 325 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
335 340 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350 355 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365 370 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380 385 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395 400 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410 415 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 44
<211> 453
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 44
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser
20 25 30

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

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

Asn Glu Lys Phe Lys Ala Arg Ala Thr Phe Thr Ala Asp Thr Ser
65 70 75

Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Phe Ser His Phe Ser Gly Ser
95 100 105

Asn Tyr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
125 130 135

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
140 145 150

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
155 160 165

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
170 175 180

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
185 190 195

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
200 205 210

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
215 220 225

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
230 235 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
275 280 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305 310 315

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
320 325 330

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
335 340 345

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
350 355 360

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
365 370 375

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
380 385 390

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
395 400 405

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
410 415 420

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
425 430 435

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
440 445 450

Pro Gly Lys

<210> 45
<211> 1042
<212> DNA
<213> Homo sapiens

<220>
<221> Unsure
<222> 447
<223> N is T or G

<400> 45
tttcctcact gactataaaa gaatagagaa ggaagggctt cagtgaccgg 50

ctgcctggct gacttacagc agtcagactc tgacaggatc atggctatga 100

tggaggtcca ggggggaccc agcctgggac agacctgcgt gctgatcgtg 150

atcttcacag tgctcctgca gtctctctgt gtggctgtaa cttacgtgta 200

ctttaccaac gagctgaagc agatgcagga caagtactcc aaaagtggca 250

ttgcttgttt cttaaaagaa gatgacagtt attgggaccc caatgacgaa 300

gagagtatga acagcccctg ctggcaagtc aagtggcaac tccgtcagct 350

cgttagaaag atgattttga gaacctctga ggaaaccatt tctacagttc 400

aagaaaagca acaaaatatt tctcccctag tgagagaaag aggtccncag 450

agagtagcag ctcacataac tgggaccaga ggaagaagca acacattgtc 500

ttctccaaac tccaagaatg aaaaggctct gggccgcaaa ataaactcct 550

gggaatcatc aaggagtggg cattcattcc tgagcaactt gcacttgagg 600

aatggtgaac tggtcatcca tgaaaaaggg ttttactaca tctattccca 650

aacatacttt cgatttcagg aggaaataaa agaaaacaca aagaacgaca 700

aacaaatggt ccaatatatt tacaaataca caagttatcc tgaccctata 750

ttgttgatga aaagtgctag aaatagttgt tggtctaaag atgcagaata 800

tggactctat tccatctatc aaggggggaat atttgagctt aaggaaaatg 850

acagaatttt tgtttctgta acaaatgagc acttgataga catggaccat 900

gaagccagtt ttttcggggc ctttttagtt ggctaactga cctggaaaga 950

aaaagcaata acctcaaagt gactattcag ttttcaggat gatacactat 1000

gaagatgttt caaaaaatct gaccaaaaca aacaaacaga aa 1042

<210> 46
<211> 281
<212> PRT
<213> Homo sapiens

<400> 46

Met	Ala	Met	Met	Glu	Val	Gln	Gly	Gly	Pro	Ser	Leu	Gly	Gln	Thr	
1				5					10						15
Cys	Val	Leu	Ile	Val	Ile	Phe	Thr	Val	Leu	Leu	Gln	Ser	Leu	Cys	
				20					25						30
Val	Ala	Val	Thr	Tyr	Val	Tyr	Phe	Thr	Asn	Glu	Leu	Lys	Gln	Met	
				35					40						45
Gln	Asp	Lys	Tyr	Ser	Lys	Ser	Gly	Ile	Ala	Cys	Phe	Leu	Lys	Glu	
				50					55						60
Asp	Asp	Ser	Tyr	Trp	Asp	Pro	Asn	Asp	Glu	Glu	Ser	Met	Asn	Ser	
				65					70						75
Pro	Cys	Trp	Gln	Val	Lys	Trp	Gln	Leu	Arg	Gln	Leu	Val	Arg	Lys	
				80					85						90
Met	Ile	Leu	Arg	Thr	Ser	Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln	Glu	
				95					100						105
Lys	Gln	Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln	
				110					115						120
Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr	Arg	Gly	Arg	Ser	Asn	Thr	
				125					130						135
Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys	Ala	Leu	Gly	Arg	Lys	
				140					145						150
Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His	Ser	Phe	Leu	Ser	
				155					160						165

Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly
170 175 180

Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu
185 190 195

Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile
200 205 210

Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser
215 220 225

Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
230 235 240

Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg
245 250 255

Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His
260 265 270

Glu Ala Ser Phe Phe Gly Ala Phe Leu Val Gly
275 280

<210> 47
<211> 411
<212> PRT
<213> Homo sapiens

<220>
<221> Xaa
<222> 410
<223> Xaa is L or M.

<400> 47
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg
1 5 10 15

Lys Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro
20 25 30

Gly Leu Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val
35 40 45

Leu Leu Leu Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp
50 55 60

Leu Ala Pro Gln Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser
65 70 75

Pro Ser Glu Gly Leu Cys Pro Pro Gly His His Ile Ser Glu Asp
80 85 90

Gly Arg Asp Cys Ile Ser Cys Lys Tyr Gly Gln Asp Tyr Ser Thr
95 100 105

His Trp Asn Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp
110 115 120

Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr
125 130 135

Val Cys Gln Cys Glu Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro
140 145 150

Glu Met Cys Arg Lys Cys Arg Thr Gly Cys Pro Arg Gly Met Val
155 160 165

Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His
170 175 180

Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
185 190 195

Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys

200 205 210

Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp
215 220 225

Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
230 235 240

Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val
245 250 255

Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly
260 265 270

Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro
275 280 285

Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
290 295 300

Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp
305 310 315

Phe Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg
320 325 330

Lys Leu Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu
335 340 345

Ala Ala Gly His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp
350 355 360

Val Asn Lys Thr Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp
365 370 375

Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu
380 385 390

Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn
395 400 405

Ala Asp Ser Ala Xaa Ser
410

<210> 48
<211> 1799
<212> DNA
<213> Homo sapiens

<400> 48
cccacgcgtc cgcataaatc agcacgcggc cggagaaccc cgcaatctct 50

gcgcccacaa aatacaccga cgatgcccga tctactttaa gggctgaaac 100

ccacgggcct gagagactat aagagcgttc cctaccgcca tggaacaacg 150

gggacagaac gccccggccg cttcgggggc ccggaaaagg cacggcccag 200

gacccaggga ggcgcgggga gccaggcctg ggctccgggt ccccaagacc 250

cttgtgctcg ttgtcgccgc ggtcctgctg ttggtctcag ctgagtctgc 300

tctgatcacc caacaagacc tagctcccca gcagagagcg gccccacaac 350

aaaagaggtc cagcccctca gagggattgt gtccacctgg acaccatatc 400

tcagaagacg gtagagattg catctcctgc aaatatggac aggactatag 450

cactcactgg aatgacctcc ttttctgctt gcgctgcacc aggtgtgatt 500

caggtgaagt ggagctaagt ccctgcacca cgaccagaaa cacagtgtgt 550

cagtgcgaag aaggcacctt ccgggaagaa gattctcctg agatgtgccg 600

gaagtgccgc acagggtgtc ccagagggat ggtcaaggtc ggtgattgta 650

caccctggag tgacatcgaa tgtgtccaca aagaatcagg catcatcata 700

ggagtcacag ttgcagccgt agtcttgatt gtggctgtgt ttgtttgcaa 750

gtctttactg tggaagaaag tccttcctta cctgaaaggc atctgctcag 800

gtggtggtgg ggaccctgag cgtgtggaca gaagctcaca acgacctggg 850

gctgaggaca atgtcctcaa tgagatcgtg agtatcttgc agcccaccca 900

ggtccctgag caggaaatgg aagtccagga gccagcagag ccaacaggtg 950

tcaacatgtt gtcccccggg gagtcagagc atctgctgga accggcagaa 1000

gctgaaaggt ctcagaggag gaggctgctg gttccagcaa atgaaggtga 1050

tcccactgag actctgagac agtgcttcga tgactttgca gacttggtgc 1100

cctttgactc ctgggagccg ctcatgagga agttgggcct catggacaat 1150

gagataaagg tggctaaagc tgaggcagcg ggccacaggg acaccttgta 1200

cacgatgctg ataaagtggg tcaacaaaac cgggcgagat gcctctgtcc 1250

acaccctgct ggatgccttg gagacgctgg gagagagact tgccaagcag 1300

aagattgagg accacttgtt gagctctgga aagttcatgt atctagaagg 1350

taatgcagac tctgccwtgt cctaagtgtg attctcttca ggaagtgaga 1400

ccttccctgg tttacctttt ttctggaaaa agcccaactg gactccagtc 1450

agtaggaaag tgccacaatt gtcacatgac cggtactgga agaaactctc 1500

ccatccaaca tcacccagtg gatggaacat cctgtaactt ttcactgcac 1550

ttggcattat ttttataagc tgaatgtgat aataaggaca ctatggaaat 1600

gtctggatca ttccgtttgt gcgtactttg agatttggtt tgggatgtca 1650

ttgttttcac agcacttttt tatcctaatg taaatgcttt atttatttat 1700

ttgggctaca ttgtaagatc catctacaaa aaaaaaaaaa aaaaaaaaag 1750

ggcggccgcg actctagagt cgacctgcag aagcttggcc gccatggcc 1799

<210> 49
<211> 440
<212> PRT
<213> Homo sapiens

<400> 49

Met	Glu	Gln	Arg	Gly	Gln	Asn	Ala	Pro	Ala	Ala	Ser	Gly	Ala	Arg	
1				5				10						15	
Lys	Arg	His	Gly	Pro	Gly	Pro	Arg	Glu	Ala	Arg	Gly	Ala	Arg	Pro	
				20				25						30	
Gly	Pro	Arg	Val	Pro	Lys	Thr	Leu	Val	Leu	Val	Val	Ala	Ala	Val	
				35				40						45	
Leu	Leu	Leu	Val	Ser	Ala	Glu	Ser	Ala	Leu	Ile	Thr	Gln	Gln	Asp	
				50				55						60	
Leu	Ala	Pro	Gln	Gln	Arg	Ala	Ala	Pro	Gln	Gln	Lys	Arg	Ser	Ser	
				65				70						75	
Pro	Ser	Glu	Gly	Leu	Cys	Pro	Pro	Gly	His	His	Ile	Ser	Glu	Asp	
				80				85						90	
Gly	Arg	Asp	Cys	Ile	Ser	Cys	Lys	Tyr	Gly	Gln	Asp	Tyr	Ser	Thr	
				95				100						105	
His	Trp	Asn	Asp	Leu	Leu	Phe	Cys	Leu	Arg	Cys	Thr	Arg	Cys	Asp	
				110				115						120	

Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr
125 130 135

Val Cys Gln Cys Glu Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro
140 145 150

Glu Met Cys Arg Lys Cys Arg Thr Gly Cys Pro Arg Gly Met Val
155 160 165

Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His
170 175 180

Lys Glu Ser Gly Thr Lys His Ser Gly Glu Ala Pro Ala Val Glu
185 190 195

Glu Thr Val Thr Ser Ser Pro Gly Thr Pro Ala Ser Pro Cys Ser
200 205 210

Leu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val Leu
215 220 225

Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys Val
230 235 240

Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp Pro
245 250 255

Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp Asn
260 265 270

Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
275 280 285

Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val
290 295 300

Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala
305 310 315

Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn
320 325 330

Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
335 340 345

Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys
350 355 360

Leu Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala
365 370 375

Ala Gly His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val
380 385 390

Asn Lys Thr Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala
395 400 405

Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp
410 415 420

His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala
425 430 435

Asp Ser Ala Met Ser
440

<210> 50
<211> 1323
<212> DNA
<213> Homo sapiens

<400> 50
atggaacaac ggggacagaa cgccccggcc gcttcggggg cccggaaaag 50

gcacggccca ggacccaggg aggcgcgggg agccaggcct gggccccggg 100

tccccaagac ccttgtgctc gttgtcgccg cggtcctgct gttggtctca 150

gctgagtctg ctctgatcac ccaacaagac ctagctcccc agcagagagc 200

ggccccacaa caaaagaggt ccagcccctc agagggattg tgtccacctg 250

gacaccatat ctcagaagac ggtagagatt gcatctcctg caaatatgga 300

caggactata gcactcactg gaatgacctc cttttctgct tgcgctgcac 350

caggtgtgat tcaggtgaag tggagctaag tccgtgcacc acgaccagaa 400

acacagtgtg tcagtgcgaa gaaggcacct tccgggaaga agattctcct 450

gagatgtgcc ggaagtgccg cacagggtgt cccagaggga tggtcaaggt 500

cggtgattgt acaccctgga gtgacatcga atgtgtccac aaagaatcag 550

gtacaaagca cagtggggaa gccccagctg tggaggagac ggtgacctcc 600

agcccaggga ctcctgcctc tccctgttct ctctcaggca tcatcatagg 650

agtcacagtt gcagccgtag tcttgattgt ggctgtgttt gtttgcaagt 700

ctttactgtg gaagaaagtc cttccttacc tgaaaggcat ctgctcaggt 750

ggtggtgggg accctgagcg tgtggacaga agctcacaac gacctggggc 800

tgaggacaat gtcctcaatg agatcgtgag tatcttgcag cccacccagg 850

tccctgagca ggaaatggaa gtccaggagc cagcagagcc aacaggtgtc 900

aacatgttgt cccccgggga gtcagagcat ctgctggaac cggcagaagc 950

tgaaaggtct cagaggagga ggctgctggt tccagcaaat gaaggtgatc 1000

ccactgagac tctgagacag tgcttcgatg actttgcaga cttggtgccc 1050

tttgactcct gggagccgct catgaggaag ttgggcctca tggacaatga 1100

gataaaggtg gctaaagctg aggcagcggg ccacagggac accttgtaca 1150

cgatgctgat aaagtgggtc aacaaaaccg ggcgagatgc ctctgtccac 1200

accctgctgg atgccttgga gacgctggga gagagacttg ccaagcagaa 1250

gattgaggac cacttgttga gctctggaaa gttcatgtat ctagaaggta 1300

atgcagactc tgccatgtcc taa 1323

<210> 51
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 51

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Gly	Gly	Val	Glu	Arg	Pro	Gly	
1				5				10						15	
Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	
				20				25						30	
Asp	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
				35				40						45	
Glu	Trp	Val	Ser	Gly	Ile	Asn	Trp	Gln	Gly	Gly	Ser	Thr	Gly	Tyr	
				50				55						60	
Ala	Asp	Ser	Val	Lys	Gly	Arg	Val	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
				65				70						75	
Lys	Asn	Ser	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	
				80				85						90	

```
Thr Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly
                 95                  100                 105

Trp Tyr Phe Asp Tyr Trp Gly Lys Gly Thr Thr Val Thr Val Ser
                110                 115                 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
                125                 130                 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
                140                 145                 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
                155                 160                 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
                200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
                215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
                290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
                350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                410                 415                 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                425                 430                 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                440                 445                 450

Lys
```

<210> 52
<211> 213
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 52
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Ser Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Ala Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Ala Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro
110 115 120

Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
125 130 135

Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
140 145 150

Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro
155 160 165

Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser
170 175 180

Leu Thr Pro Glu Gln Trp Lys Ser His Lys Ser Tyr Ser Cys Gln
185 190 195

Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr
200 205 210

Glu Cys Ser

<210> 53
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 53
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Glu Arg Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
20 25 30

Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr
50 55 60

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

HFF 157

65 70 75

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly
95 100 105

Trp Tyr Phe Asp Leu Trp Gly Lys Gly Thr Thr Val Thr Val Ser
110 115 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
170 175 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185 190 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200 205 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215 220 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
320 325 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
335 340 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350 355 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365 370 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380 385 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395 400 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410 415 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 54
<211> 213
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 54
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro
110 115 120

Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
125 130 135

Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
140 145 150

Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro
155 160 165

Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser
170 175 180

Leu Thr Pro Glu Gln Trp Lys Ser His Lys Ser Tyr Ser Cys Gln
185 190 195

Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr
200 205 210

Glu Cys Ser

<210> 55
<211> 126
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 55
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Glu Arg Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
20 25 30

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

Trp Val Ser Gly Ile Asn Trp Gln Gly Gly Ser Thr Gly Tyr Ala
50 55 60

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

Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
80 85 90

Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly Trp
95 100 105

Tyr Phe Asp Tyr Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro
125

<210> 56
<211> 108
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 56
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Ser Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Ala Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Ala Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly

<210> 57
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 57
Arg Ala Ser Ser Ser Val Ser Tyr Met His
5 10

<210> 58
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 58
Ala Pro Ser Asn Leu Ala Ser
5

<210> 59

<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 59
Gln Gln Trp Ser Phe Asn Pro Pro Thr
5

<210> 60
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 60
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
5 10

<210> 61
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 61
Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
1 5 10 15

Lys Gly

<210> 62
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 62
Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
5 10

<210> 63
<211> 213
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 63
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

```
Ser Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
                95                  100                 105

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
            110                 115                 120

Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
            125                 130                 135

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
            140                 145                 150

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
            155                 160                 165

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
            170                 175                 180

Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
            185                 190                 195

Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
            200                 205                 210

Gly Glu Cys
```

<210> 64
<211> 213
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 64

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
            20                  25                  30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
            35                  40                  45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
            50                  55                  60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
            65                  70                  75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
            80                  85                  90

Ala Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            95                  100                 105

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
            110                 115                 120

Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
            125                 130                 135

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
            140                 145                 150

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
            155                 160                 165

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
            170                 175                 180

Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
            185                 190                 195
```

Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
200 205 210

Gly Glu Cys

<210> 65
<211> 452
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 65
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His

305 310 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
320 325 330

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
335 340 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
350 355 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
365 370 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
380 385 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
395 400 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
410 415 420

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

<210> 66
<211> 452
<212> PRT
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<220>
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Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305 310 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
320 325 330

Ala Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys
335 340 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
350 355 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
365 370 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
380 385 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
395 400 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
410 415 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
440 445 450

Gly Lys

<210> 67
<211> 10
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<222> 9
<223> Xaa is M or L.

<400> 67
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<210> 68

<211> 9
<212> PRT
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<220>
<223> Sequence is synthesized.

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<210> 69
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<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

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<221> Xaa
<222> 8
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<210> 70
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

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<221> Xaa
<222> 6
<223> Xaa is N, A, Y, W or D.

<220>
<221> Xaa
<222> 7
<223> Xaa is S or R.

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<210> 71
<211> 451
<212> PRT
<213> Artificial sequence

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20 25 30

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

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Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
                50                  55                  60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
                95                 100                 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
               110                 115                 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
               125                 130                 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
               140                 145                 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
               155                 160                 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
               170                 175                 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
               185                 190                 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
               200                 205                 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
               215                 220                 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
               230                 235                 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
               245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
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Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
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Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
               290                 295                 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
               305                 310                 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
               320                 325                 330

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
               335                 340                 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
               350                 355                 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
               365                 370                 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
               380                 385                 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
               395                 400                 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
               410                 415                 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
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425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
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Gly

<210> 72
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

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Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

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Tyr Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
                305                 310                 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
                320                 325                 330

Ala Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys
                335                 340                 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
                350                 355                 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                365                 370                 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
                380                 385                 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                395                 400                 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                410                 415                 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
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Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
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<210> 73
<211> 107
<212> PRT
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<220>
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<400> 73

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                20                  25                  30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
                35                  40                  45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
                50                  55                  60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                65                  70                  75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
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Ala Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
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Lys Arg
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<210> 74
<211> 122
<212> PRT
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<220>
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<400> 74

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                20                  25                  30

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
                50                  55                  60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
                95                 100                 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
               110                 115                 120

Ser Ser
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170

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(71) Solicitante *(para todos los países destinatarios con excepción de US)*: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

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(81) Países designados *(a menos que se indique lo contrario para cada clase de protección disponible)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NO, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Países designados *(a menos que se indique lo contrario para cada clase de protección disponible)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Título: FORMULACIÓN DE ANTICUERPO EN AMORTIGUADOR DE ACETATO DE HISTIDINA

(57) Extracto: La presente solicitud describe formulaciones de anticuerpo, incluidos anticuerpos monoclonales formulados en amortiguador de acetato de histidina, así como louna formulación que comprende un anticuerpo que se une al dominio II de HER2 (por ejemplo, Pertuzumab), y una formulación que comprende un anticuerpo que se une a DR5 (por ejemplo, Apomab).

WO 2006/044908 A3

FORMULACIONES DE ANTICUERPO

Ésta es una solicitud no provisional presentada bajo 37 CFR 1.53(b) que reivindica prioridad a la solicitud provisional 60/620,413 presentada el 20 de octubre de 2004, cuyo contenido se incorpora aquí por referencia.

Campo de la Invención

La presente invención concierne a formulaciones de anticuerpo, incluidos anticuerpos monoclonales formulados en amortiguador de acetato de histidina, así como a una formulación que comprende un anticuerpo que se une al dominio II de HER2 (por ejemplo, Pertuzumab), y a una formulación que comprende un anticuerpo que se une a DR5 (por ejemplo, Apomab).

Antecedentes de la Invención

En los diez últimos años, los avances en biotecnología han hecho posible producir una variedad de proteínas para aplicaciones farmacéuticas utilizando técnicas de ADN recombinante. Debido a que las proteínas son más grandes y más complejas que los medicamentos orgánicos e inorgánicos tradicionales (es decir, que poseen múltiples grupos funcionales además de estructuras tridimensionales complejas), la formulación de tales proteínas plantea problemas especiales. Para que una proteína permanezca biológicamente activa, una formulación tiene que preservar intacta la integridad de conformación de por lo menos una secuencia central de aminoácidos de la proteína, protegiendo al mismo tiempo contra la degradación los múltiples grupos funcionales de la proteína. Las rutas de degradación para las proteínas pueden involucrar inestabilidad química (es decir, cualquier proceso que implique modificación de la proteína mediante formación de enlaces o corte proteolítico resultante en una nueva entidad química), o inestabilidad física (*es decir*, cambios en la estructura de mayor orden de la proteína). La inestabilidad química puede resultar de desamidación, racemización, hidrólisis, oxidación, eliminación beta o intercambio de disulfuros. La inestabilidad física puede resultar de

desnaturalización, agregación, precipitación y adsorción, por ejemplo. Las tres rutas más comunes de degradación de las proteínas son la agregación, desamidación y oxidación de proteínas. Cleland *et al., Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993).

Formulaciones de Anticuerpo

Los anticuerpos forman parte de las proteínas usadas para aplicaciones farmacéuticas. Un ejemplo de un anticuerpo útil para terapia es un anticuerpo que se une al antígeno HER2, tal como Pertuzumab.

La Patente de los Estados Unidos No. 6,339,142 describe una composición de anticuerpo HER2, que comprende una mezcla de anticuerpo anti-HER2 y una o más variantes ácidas del mismo, en donde la cantidad de la(s) variante(s) ácida(s) es menos de alrededor del 25%. Un anticuerpo HER2 ejemplar es Trastuzumab.

Las Patentes de los Estados Unidos No. 6,267,958 y 6,685,940 (Andya *et al.*) describen formulaciones liofilazadas de anticuerpo, incluidas formulaciones de anticuerpo HER2 e IgE. WO97/04807 y US 2004/0197326A1 (Fick *et al.*) describen métodos para tratar asma alérgico con un anticuerpo IgE. WO99/01556 (Lowman *et al.*) se refiere a un anticuerpo IgE con residuos aspartilo propensos a isomerización, y variantes mejoradas del mismo. US 2002/0045571 (Liu *et al.*) provee formulaciones concentradas de proteína de viscosidad reducida, ejemplificadas por formulaciones del anticuerpo IgE humanizado, rhuMAb E25 y E26. WO 02/096457 y US 2004/0170623 (Arvinte *et al.*) describen formulaciones líquidas estables que comprenden el anticuerpo anti-IgE E25. Véase, además, US 2004/0197324 Al (Liu y Shire) concerniente a una formulación anti-IgE de concentración elevada.

La Patente de los Estados Unidos No. 6,171,586 (Lam *et al.*) describe formulaciones estables acuosas de anticuerpo. Un anticuerpo F(ab')2 rhuMAb CD 18 se formuló en amortiguadores de acetato de sodio e histidina-HCl. La formulación preferida para rhuMAb CD18 fue 10mM de acetato de sodio, 8% trehalosa, 0.01% TWEEN 20™, pH 5.0. Las formulaciones en acetato (pH 5.0) de rhuMAb CD20 almacenadas a 40°

durante un mes demostraron una mayor estabilidad que aquellas muestras formuladas en histidina (pH 5.0 ó 6.0).

US 2003/0190316 (Kakuta *et al.*) concierne al anticuerpo hPM-1 formulado, un anticuerpo de receptor IL-6 humanizado. La pérdida de monómero fue máxima en citrato de sodio (pH 6.7), seguida por fosfato de sodio (pH 6.8), Tris-HCl (pH 7.2), histidina-HCl (pH 7.2) y glicina (pH 7.6) en orden descendente. Se evaluó el efecto de fosfato-Na (pH 6.5), fosfato-His (pH 6.0 o 6.5), His-HCl (pH 6.5), y fosfato-Na (pH 6.0) sobre la estabilidad de hPM-1.

WO2004/071439 (Burke *et al.*) establece que surgieron impurezas en una formulación de natalizumab (anticuerpo monoclonal humanizado de integrina anti-alpha4) de la degradación de polisorbato 80, aparentemente a través de una reacción de oxidación que implicaba iones de metal e histidina. Es consecuencia, se seleccionó un amortiguador de fosfato.

WO 2000/066160 (contraparte en idioma inglés de EP 1 174 148A1) (Okada *et al.*) se refiere a una formulación de anticuerpo C4G1 humanizado, que se une a un receptor de fibrinógeno de una glucoproteína de membrana plaquetaria humana GPIIb/IIIa, en un amortiguador de fosfato de sodio o citrato de sodio.

WO2004/019861 (Johnson *et al.*) concierne a CDP870, un fragmento Fab anti-TNFα peglilado, formulado a 200mg/ml en 50mM de acetato de sodio (pH 5.5) y 125mM de cloruro de sodio.

WO2004/004639 (Nesta, P.) se refiere a una formulación para huC242-DM1, una inmunotoxina activada por tumor, en 50mM de un amortiguador de ácido succínico (pH 6.0) y sacarosa (5% p/v).

WO03/039485 (Kaisheva *et al.*) descubrió que Daclizumab (un anticuerpo de receptor EL-2 a humanizado) tenía la máxima estabilidad en amortiguador de succinato de sodio a pH 6.0, y perdía potencia rápidamente en histidina, a medida que el amortiguador se oxidaba.

WO 2004/001007 concierne a un anticuerpo monoclonal CD80 en un amortiguador de HCl de histidina, acetato de sodio o citrato de sodio.

La Patente de los Estados Unidos No. 6,252,055 (Relton, J.) se refiere a anticuerpos anti-CD4 y anti-CD23 formulados en amortiguadores de maleato, succinato, acetato de sodio o fosfato, indentificándose el fosfato como el amortiguador preferido.

La Patente de los Estados Unidos No. 5,608,038 (Eibl *et al.*) se refiere a preparaciones altamente concentradas de inmunoglobulina policlonal con inmunoglobulina, glucosa o sacarosa, y cloruro de sodio contenido en ellas.

WO03/015894 (Oliver *et al.*) se refiere a una formulación acuosa de 100mg/mL SYNAGIS®, 25mM histidina-HCl, 1.6mM glicina, pH 6.0, y una SYNAGIS® liofilizada la cual, cuando se formula (antes de la liofilización), contiene 25 mM de histidina, 1.6mM de glicina y 3% p/v de mannitol a pH 6.0.

US 2004/0191243 Al (Chen *et al.*) divulga una formulación de ABX-IL8, un anticuerpo IgG2 humano.

US 2003/0113316 Al (Kaisheva *et al.*) se refiere a una formulación liofilizada de anticuerpo de receptor anti-IL2.

Anticuerpos HER2

La familia HER de tirosina cinasas de receptor son importantes mediadores del crecimiento, diferenciación y supervivencia celular. La familia de receptores incluye cuatro miembros distintos incluido el receptor del factor de crecimiento epidérmico (EGFR, ErbBl, o HER1), HER2 (ErbB2 o p185^{neu}), HER3 (ErbB3) y HER4 (ErbB4 o tyro2).

EGFR, codificado por el gen erbB1, ha sido casualmente implicado en neoplasia maligna humana. En particular, se ha observado una expresión cada vez mayor de EGFR en cáncer de mama, vejiga, pulmón, cabeza, cuello y estómago, así como en glioblastomas. La creciente expresión del receptor EGFR se asocia a menudo con una producción en aumento del ligando EGFR, factor α de crecimiento transformante (TGF-α), por las mismas células tumorales resultante en una activación del receptor por una ruta estimuladora autocrina. Baselga and Mendelsohn *Pharmac. Ther.* 64:127-154 (1994). Anticuerpos monoclonales dirigidos contra el EGFR o

sus ligandos, TGF-α y EGF, han sido evaluados como agentes terapéuticos en el tratamiento de tales neoplasias malignas. Véase, por ejemplo, Baselga and Mendelsohn., *supra*; Masui *et al. Cancer Research* 44:1002-1007 (1984); y Wu *et al. J. Clin. Invest.* 95: 1897-1905 (1995).

El segundo miembro de la familia HER, p185^{neu}, se identificó originalmente como el producto del gen transformante de neuroblastomas de ratas tratadas químicamente. La forma activada del protooncogén *neu* resulta de una mutación puntual (valina en ácido glutámico) en la región transmembrana de la proteína codificada. La amplificación del homólogo humano de *neu* se observa en neoplasias malignas de mama y ovario y se correlaciona con una prognosis deficiente (Slamon *et al.*, *Science*, 235: 177-182 (1987); Slamon *et al.*, *Science*, 244:707-712 (1989); y Patente de los Estados Unidos No. 4,968,603). A la fecha, no se ha informado una mutación puntual análoga a la hallada en el protooncogén *neu* para tumores humanos. La sobreexpresión de HER2 (con frecuencia, pero no uniformemente, debida a amplificación génica) se ha observado igualmente en otros carcinomas, incluidos carcinomas del estómago, endometrio, glándula salival, pulmón, riñón, colon, tiroides, páncreas y vejiga. Véase, entre otros, King *et al.*, *Science*, 229:91 A (1985); Yokota *et al.*, *Lancet*: 1:765-767 (1986); Fukushige *et al.*, *Mol Cell Biol*, 6:955-958 (1986); Guerin *et al.*, *Oncogene Res.*, 3:21-31 (1988); Cohen *et al.*, *Oncogene*, 4:81-88 (1989); Yonemura *et al.*, *Cancer Res.*, 51: 1034 (1991); Borst *et al.*, *Gynecol. Oncol.*, 38:364 (1990); Weiner *et al.*, *Cancer Res.*, 50:421-425 (1990); Kern *et al.*, *Cancer Res.*, 50:5184 (1990); Park *et al.*, *Cancer Res.*, 49:66O5 (1989); Zhau *et al.*, *Mol. Carcinog.*, 3:254-257 (1990); Aasland *et al.*, *Br. J. Cancer* 57:358-363 (1988); Williams *et al.*, *Pathobiology* 59:46-52 (1991); y McCann *et al.*, *Cancer*, 65:88-92 (1990). HER2 puede sobreexpresarse en cancer de próstata (Gu *et al.*, *Cancer Lett.* 99:185-9 (1996); Ross *et al.*, *Hum. Pathol.* 28:827-33 (1997); Ross *et al.*, *Cancer* 79:2162-70 (1997); y Sadasivan *et al.*, *Urol.* 150:126-31 (1993)).

Se han descrito anticuerpos dirigidos contra el 185^{neu} de rata y productos de proteína HER2 humana. Drebin y colegas han cultivado

anticuerpos contra el producto génico *neu* de rata, p185^{neu}. Véase, por ejemplo, Drebin *et al., Cell* 41:695-706 (1985); Myers *et al., Meth. Enzym.* 198:277-290 (1991); y WO94/22478. Drebin *et al., Oncogene* 2:273-277 (1988) informan que mezclas de anticuerpos que reaccionan con dos regiones distintas de p185^{neu} resultan en efectos sinérgicos anti-tumorales sobre células NIH-3T3 transformadas con *neu*, implantadas en ratones inmunitariamente desprotegidos. Véase también la Patente de los Estados Unidos 5,824,311 expedida el 20 de octubre de 1998.

Hudziak *et al., Mol. Cell. Biol.* 9(3): 1165-1172 (1989) describen la generación de panel de anticuerpos HER2, que se caracterizaron usando la línea celular SK-BR-3 de tumor de mama humano. La proliferación celular relativa de las células SK-BR-3, luego de la exposición a los anticuerpos, se determinó mediante tinción con cristales de violeta de las monocapas después de 72 horas. Usando este análisis, se obtuvo la máxima inhibición con el anticuerpo denominado 4D5, el cual inhibió la proliferación celular en 56%. Otros anticuerpos en el panel redujeron la proliferación celular en una magnitud menor en este análisis. Se encontró además que el anticuerpo 4D5 sensibilizaba las líneas celulares de tumor de mama, que sobreexpresaban HER2, a los efectos citotóxicos de TNF-α. Véase también la Patente de los Estados Unidos No. 5,677,171 expedida el 14 de octubre de 1997. Los anticuerpos HER2, discutidos en Hudziak *et al.*, se caracterizan igualmente en Fendly *et al., Cancer Research* 50:1550- 1558 (1990); Kotts *et al., In Vitro* 26(3):59A (1990); Sarup *et al., Growth Regulation* 1:72-82 (1991); Shepard *et al., J. Clin. Immunol.* 11(3): 117-127 (1991); Kumar *et al., Mol. Cell. Biol.* 11(2):979-986 (1991); Lewis *et al., Cancer Immunol. Immunother.* 37:255-263 (1993); Pietras *et al., Oncogene* 9:1829-1838 (1994); Vitetta *et al., Cancer Research* 54:5301-5309 (1994); Sliwkowski *et al., J. Biol. Chem.* 269(20): 14661-14665 (1994); Scott *et al., J. Biol. Chem.* 266:14300-5 (1991); D'Souza *et al., Proc. Natl. Acad. Sci.* 91:7202-7206 (1994); Lewis *et al., Cancer Research* 56:1457-1465 (1996); y Schaefer *et al., Oncogene* 15:1385-1394 (1997). Una versión humanizada recombinante del anticuerpo HER2 murino 4D5 (huMAb4-D5-8, rhuMAb

HER2, Trastuzumab o HERCEPTIN®; Patente de los Estados Unidos No. 5,821,337) es clínicamente activa en pacientes con neoplasias malignas de mama metastásicas que sobreexpresan HER2, los cuales han recibido terapia previa extensiva anti-cáncer (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 (1996)). El Trastuzumab recibió aprobación de comercialización de la Dirección de Alimentos y Drogas de los Estados Unidos el 25 de septiembre de 1998 para el tratamiento de pacientes con cáncer metastático de mama, cuyos tumores sobreexpresan la proteína HER2.

Otros anticuerpos HER2 con varias propiedades se han descrito en Tagliabue *et al.*, *Int. J. Cancer* 47 :933-937 (1991); McKenzie *et al.*, *Oncogene* 4:543-548 (1989); Maier *et al.*, *Cancer Res.* 51:5361-5369 (1991); Bacus *et al.*, *Molecular Carcinogenesis* 3:350-362 (1990); Stancovski *et al.*, *PNAS (USA)* 88:8691-8695 (1991); Bacus *et al.*, *Cancer Research* 52:2580-2589 (1992); Xu *et al.*, *Int. J. Cancer* 53:401-408 (1993); WO94/00136; Kasprzyk *et al.*, *Cancer Research* 52:2771-2776 (1992); Hancock *et al.*, *Cancer Res.* 51:4575-4580 (1991); Shawver *et al.*, *Cancer Res.* 54:1367-1373 (1994); Arteaga *et al.*, *Cancer Res.* 54:3758-3765 (1994); Harwerth *et al.*, *J. Biol. Chem.* 267:15160-15167 (1992); Patente de los Estados Unidos No. 5,783,186; y Klapper *et al.*, *Oncogene* 14:2099-2109 (1997).

El cribado de la homología ha resultado en la identificación de dos otros miembros de la familia del receptor HER; HER3 (Patentes de los Estados Unidos No. 5,183,884 y 5,480,968, así como Kraus *et al.*, *PNAS (USA)* 86:9193-9197 (1989)) y HER4 (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)). Estos dos receptores exhiben una expresión cada vez mayor en por lo menos algunas líneas celulares de cáncer de mama.

Los receptores HER se encuentran generalmente en varias combinaciones en células, y se cree que la heterodimerización incrementa la diversidad de las respuestas celulares a una variedad de ligandos HER (Earp *et al.*, *Breast Cancer Research and Treatment* 35: 115-132 (1995)). EGFR

se une por seis ligandos diferentes; factor de crecimiento epidérmico (EGF), factor α de crecimiento transformante (TGF-α), anfirregulina, factor de crecimiento epidérmico de unión a heparina (HB-EGF), betacelulina y epirregulina (Groenen *et al., Growth Factors* 11:235-257 (1994)). Una familia de proteínas de herregulina, resultantes del corte y empalme alternativo de un solo gen, son ligandos para HER3 y HER4. La familia de herregulina incluye las herregulinas α, β y γ (Holmes *et al., Science*, 256:1205-1210 (1992); Patente de los Estados Unidos No. 5,641,869; y Schaefer *et al., Oncogene* 15:1385-1394 (1997)); factores de diferenciación *neu* (NDFs), factores de crecimiento glial (GGFs); actividad inductora del receptor de acetilcolina (ARIA); y factor derivado de neuronas motoras y sensoras (SMDF). Para una reseña, véase Groenen *et al., Growth Factors* 11:235-257 (1994); Lernke, G. *Molec. & Cell. Neurosci.* 7:247-262 (1996) y Lee *et al., Pharm. Rev.* 47:51-85 (1995). Recientemente se identificaron tres ligandos HER adicionales; neurregulina-2 (NRG-2), de la que se informa que se une a HER3 ó HER4 (Chang *et al., Nature* 387 509-512 (1997); y Carraway *et al., Nature* 387:512-516 (1997)); neurregulina-3 que une HER4 (Zhang *et al., PNAS (USA)* 94(18):9562-7 (1997)); y neurregulina-4 que une HER4 (Harari *et al., Oncogene* 18:2681-89 (1999)). HB-EGF, betacelulina y epirregulina se unen asimismo a HER4.

Aunque EGF y TGFα no unen HER2, EGF estimula a EGFR y a HER2 a formar un heterodímero, el cual activa EGFR y resulta en transfosforilación de HER2 en el heterodímero. La dimerización y/o transfosforilación parecen activar la tirosina cinasa HER2. Véase Earp *et al., supra.* Análogamente, cuando HER3 se co-expresa con HER2, se forma un complejo de señalización activo, y anticuerpos dirigidos contra HER2 son capaces de alterar este complejo (Sliwkowski *et al., J. Biol. Chem.*, 269(20): 14661-14665 (1994)). Adicionalmente, se incrementa la afinidad de HER3 para la herregulina (HRG) a un estado de mayor afinidad cuando se co-expresa con HER2. Véase también, Levi *et al., Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al., Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); y Lewis *et al., Cancer Res.*, 56: 1457-1465

(1996) con respecto al complejo de proteínas HER2-HER3. HER4, como HER3, forma un complejo de señalización activo con HER2 (Carraway y Cantley, *Cell* 78:5-8 (1994)).

Para acceder a la ruta de señalización HER, rhuMAb 2C4 (Pertuzumab, OMNITARG™) se desarrolló como un anticuerpo humanizado que inhibe la dimerización de HER2 con otros receptores HER, inhibiendo en esa forma la fosforilación y activación desencadenadas por ligando, y la activación corriente abajo de las rutas RAS y AKT. En un estudio clínico de fase I de Pertuzumab como un agente único para tratar tumores sólidos, se trataron con Pertuzumab 3 pacientes con cáncer avanzado de ovarios. Una tuvo una respuesta parcial duradera, y una paciente adicional tuvo una enfermedad estable durante 15 semanas, Agus *et al., Proc Am. Soc. Clin. Oncol.* 22: 192, Abstract 771 (2003).

Anticuerpos DR5

En el arte se han identificado varios ligandos y receptores pertenecientes a la superfamilia del factor de necrosis tumoral (TNF). Incluidos entre tales ligandos se encuentran el factor alfa de necrosis tumoral ("TNF-alfa"), el factor beta de necrosis tumoral ("TNF-beta" o "linfotoxina-alfa"), linfotoxina-beta ("LT-beta"), ligando CD30, ligando CD27, ligando CD40, ligando OX-40, ligando 4-1BB, LIGHT, ligando Apo-1 (también denominado ligando Fas o ligando CD95), ligando Apo-2 (también denominado Apo2L ó TRAIL), ligando Apo-3 (también denominado TWEAK), APRIL, ligando OPG (también denominado ligando RANK, ODF, o TRANCE), y TALL-1 (también denominado BlyS, BAFF ó THANK) (Véase, *por ejemplo*, Ashkenazi, *Nature Review*, 2:420-430 (2002); Ashkenazi and Dixit, *Science*, 281:1305-1308 (1998); Ashkenazi and Dixit, *Curr. Opin. Cell Biol.*, 11:255-260 (2000); Golstein, *Curr. Biol.*, 7:750-753 (1997) Wallach, *Cytokine Reference*, Academic Press, 2000, pages 377-411; Locksley *et al.*, *Cell*, 104:487-501 (2001); Gruss and Dower, *Blood*, 85:3378-3404 (1995); Schmid *et al.*, *Proc. Natl. Acad. Sci.*, 83:1881 (1986); Dealtry *et al.*, *Eur. J. Immunol.*, 17:689 (1987); Pitti *et al.*, *J. Biol. Chem.*, 271:12687-12690 (1996); Wiley *et al.*, *Immunity*, 3:673-682

(1995); Browning *et al.*, *Cell*, 72:847-856 (1993); Armitage *et al.*, *Nature*, 357:80-82 (1992); WO 97/01633 publicada el 16 de enero de 1997; WO 97/25428 publicada el 17 de julio de 1997; Marsters *et al.*, *Curr. Biol.*, 8:525-528 (1998); Chicheportiche *et al.*, *Biol. Chem.*, 272:32401-32410 (1997); Hahne *et al.*, *J. Exp. Med.*, 188:1185-1190 (1998); WO98/28426 publicada el 2 de julio de 1998; WO98/46751 publicada el 22 de octubre de 1998; WO/98/18921 publicada el 7 de mayo de 1998; Moore *et al.*, *Science*, 285:260-263 (1999); Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); Schneider *et al.*, *J. Exp. Med.*, 189:1747-1756 (1999); Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274:15978-15981 (1999)).

La inducción de varias respuestas celulares, mediadas por tales ligandos de la familia TNF, se inicia típicamente por su unión a receptores celulares específicos. Algunos de los ligandos, pero no todos, de la familia TNF se unen a, e inducen diferente actividad biológica a través de, "receptores de muerte" de superficie celular para activar caspasas, o enzimas que llevan a cabo la vía de muerte celular o apoptosis (Salvesen *et al.*, *Cell*, 91:443-446 (1997)). Incluidos entre los miembros de la superfamilia de receptores TNF, identificados hasta la fecha, se encuentran TNFR1, TNFR2, TACI, GITR, CD27, OX-40, CD30, CD40, HVEM, Fas (también denominado Apo-1 o CD95), DR4 (también denominado TRAIL-R1), DR5 (también denominado Apo-2 o TRAIL-R2), DcR1, DcR2, osteoprotegerina (OPG), RANK y Apo-3 (también denominado DR3 o TRAMP).

La mayoría de estos miembros de la familia de receptores TNF comparten la estructura típica de los receptores de superficie celular, incluidas las regiones extracelulares, de transmembrana e intracelulares, mientras que otros se encuentran naturalmente como proteínas solubles, que carecen de un dominio transmembrana e intracelular. La porción extracelular de los TNFR típicos contiene un patrón repetitivo de secuencia de aminoácidos de múltiples dominios, ricos en cisteína (CRD), que se inician a partir del término NH_2.

El ligando designado como Apo-2L o TRAIL se identificó hace varios años como un miembro de la familia TNF de citocinas, (véase *por ejemplo*, Wiley *et al.*, *Immunity*, 3:673-682 (1995); Pitti *et al.*, *J. Biol. Chem.*, 271:12697-12690 (1996); WO 97/01633; WO 97/25428; Patente de los Estados Unidos 5,763,223 expedida el 9 de junio de 1998; Patente de los Estados Unidos 6,284,236 expedida el 4 de septiembre de 2001). El polipéptido Apo2L/TRAIL humano de secuencia natural, de longitud total, es una proteína trasmembrana tipo II, de 281 aminoácidos de longitud. Algunas células pueden producir una forma soluble natural del polipéptido, mediante corte proteolítico enzimático de la región extracelular del polipéptido (Mariani *et al.*, *J. Cell. Biol.*, 137:221-229 (1997)). Estudios cristalográficos de formas solubles de Apo2L/TRAIL revelan una estructura homotrimérica similar a las estructuras de TNF y otras proteínas relacionadas (Hymowitz *et al.*, *Molec. Cell*, 4:563-571 (1999); Cha *et al.*, *Immunity*, 11:253-261 (1999); Mongkolsapaya *et al.*, *Nature Structural Biology*, 6:1048 (1999); Hymowitz *et al.*, *Biochemistry*, 39:633-644 (2000)). Sin embargo, se descubrió que Apo2L/TRAIL, a diferencia de otros miembros de la familia TNF, posee una característica estructural única, en la que tres residuos de cisteína (en la posición 230 de cada subunidad en el homotrímero) coordinan conjuntamente un átomo de zinc, y que la unión de zinc es importante para la estabilidad y actividad biológica del trímero (Hymowitz *et al.*, *supra*; Bodmer *et al.*, *J. Biol. Chem.*, 275:20632-20637 (2000)).

Se ha informado en la literatura que Apo2L/TRAIL puede jugar un papel en la modulación del sistema inmunitario, incluidas enfermedades autoinmunitarias tales como artritis reumatoide (véase *por ejemplo*, Thomas *et al.*, *J. Immunol.*, 161:2195-2200 (1998); Johnsen *et al.*, *Cytokine*, 11:664-672 (1999); Griffith *et al.*, *J. Exp. Med.*, 189:1343-1353 (1999); Song *et al.*, *J. Exp. Med.*, 191:1095-1103 (2000)).

Se ha divulgado asimismo que formas solubles de Apo2L/TRAIL inducen apoptosis en una variedad de células cancerosas, incluidos tumores de colon, pulmón, mama, próstata, vejiga, riñón, ovarios y cerebro, así

como melanoma, leucemia, y mieloma múltiple (véase *por ejemplo*, Wiley *et al.*, *supra*; Pitti *et al.*, *supra*; Patente de los Estados Unidos 6,030,945 expedida el 29 de febrero de 2000; Patente de los Estados Unidos 6,746,668 expedida el 8 de junio de 2004; Rieger *et al.*, *FEBS Letters*, 427:124-128 (1998); Ashkenazi *et al.*, *J. Clin. Invest.*, 104:155-162 (1999); Walczak *et al.*, *Nature Med.*, 5:157-163 (1999); Keane *et al.*, *Cancer Research*, 59:734-741 (1999); Mizutani *et al.*, *Clin. Cancer Res.*, 5:2605-2612 (1999); Gazitt, *Leukemia*, 13:1817-1824 (1999); Yu *et al.*, *Cancer Res.*, 60:2384-2389 (2000); Chinnaiyan *et al.*, *Proc. Natl. Acad. Sci.*, 97:1754-1759 (2000)]. Estudios *in vivo* en modelos de tumor murino sugieren además que Apo2L/TRAIL, solo o en combinación con quimioterapia o terapia de radiación, puede ejercer efectos anti-neoplásicos sustanciales (véase *por ejemplo*, Ashkenazi *et al.*, *supra*; Walzcak *et al.*, *supra*; Gliniak *et al.*, *Cancer Res.*, 59:6153-6158 (1999); Chinnaiyan *et al.*, *supra*; Roth *et al.*, *Biochem. Biophys. Res. Comm.*, 265:1999 (1999); Solicitud PCT US/00/15512; Solicitud PCT US/01/23691). En contraste con muchos tipos de células cancerosas, la mayoría de los tipos de células humanas normales parecen ser resistentes a la inducción de apoptosis por ciertas formas recombinantes de Apo2L/TRAIL (Ashkenazi *et al.*, *supra*; Walzcak *et al.*, *supra*). Jo *et al.* han divulgado que una forma soluble, marcada con polihistidina, de Apo2L/TRAIL indujo apoptosis *in vitro* en hepatocitos humanos aislados normales, más no en hepatocitos no humanos, (Jo *et al.*, *Nature Med.*, 6:564-567 (2000); véase también, Nagata, *Nature Med.*, 6:502-503 (2000)). Se cree que ciertas preparaciones de Apo2L/TRAIL recombinantes pueden variar en términos de propiedades bioquímicas y actividades biológicas en células enfermas versus células normales, dependiendo, por ejemplo, de la presencia o ausencia de una molécula marcadora, el contenido de zinc, y el % de contenido de trímero (véase Lawrence *et al.*, *Nature Med.*, Letter to the Editor, 7:383-385 (2001); Qin *et al.*, *Nature Med.*, Letter to the Editor, 7:385-386 (2001)).

Se ha descubierto que Apo2L/TRAIL une por lo menos cinco receptores diferentes. Por lo menos dos de los receptores, que unen

Apo2L/TRAIL, contienen un dominio de muerte celular citoplásmico funcional. Un receptor semejante se ha designado "DR4" (y, alternativamente, TR4 o TRAIL-R1) (Pan *et al.*, *Science*, 276:111-113 (1997); véase también WO98/32856 publicada el 30 de julio de 1998; WO99/37684 publicada el 29 de julio de 1999; WO 00/73349 publicada el 7 de diciembre de 2000; US 6,433,147 expedida el 13 de agosto de 2002; US 6,461,823 expedida el 8 de octubre de 2002, y US 6,342,383 expedida el 29 de enero de 2002).

Otro receptor similar para Apo2L/TRAIL se ha denominado DR5 (alternativamente, se ha designado también Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 ó KILLER (véase, por ejemplo, Sheridan *et al.*, *Science*, 277:818-821 (1997); Pan *et al.*, *Science*, 277:815-818 (1997); WO98/51793 publicada el 19 de noviembre de 1998; WO98/41629 publicada el 24 de septiembre de 1998; Screaton *et al.*, *Curr. Biol.*, 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 publicada el 20 de agosto de 1998; EP870,827 publicada el 14 de octubre de 1998, WO98/46643 publicada el 22 de octubre de 1998; WO99/02653 publicada el 21 de enero de 1999; WO99/09165 publicada el 25 de febrero de 1999, WO99/11791 publicada el 11 de marzo de 1999; US 2002/0072091 publicada el 13 de agosto de 2002; US 2002/0098550 publicada el 7 de diciembre de 2001; US 6,313,269 expedida el 6 de diciembre de 2001; US 2001/0010924 publicada el 2 de agosto de 2001; US 2003/01255540 publicada el 3 de julio de 2003; US 2002/0160446 publicada el 31 de octubre de 2002; US 2002/0048785 publicada el 25 de abril 2002; US 6,342,369 expedida en febrero de 2002; US 6,569,642 expedida el 27 de mayo de 2003; US 6,072,047 expedida el 6 de junio de 2000; US 6,642,358 expedida el 4 de noviembre de 2003; US 6,743,625 expedida el 1 de junio de 2004). Al igual que DR4, se divulga que DR5 contiene un dominio de muerte celular citoplásmico y es capaz de señalizar apoptosis tras la unión del ligando (o tras de unir una molécula, tal como un anticuerpo agonista, que imita la actividad del ligando. La estructura cristalina del complejo

formado entre Apo-2L/TRAIL y DR5 se describe en Hymowitz *et al.*, *Molecular Cell*, 4:563-571 (1999).

Luego de la unión del ligando, los dos DR4 y DR5 pueden desencadenar apoptosis independientemente, reclutando y activando el iniciador de apoptosis, caspasa-8, a través de la molécula de adaptador que contiene el dominio de muerte celular, denominado FADD/Mort1 (Kischkel et al., *Immunity*, 12:611-620 (2000); Sprick *et al.*, *Immunity*, 12:599-609 (2000); Bodmer *et al.*, *Nature Cell Biol.*, 2:24:1-24:3 (2000)).

Se ha divulgado que Apo2L/TRAIL une también aquellos receptores designados DcR1, DcR2 y OPG, los cuales se cree que funcionan como inhibidores, más bien que como transductores de señalización (véase, por ejemplo, DCR1 (también de nominado TRID, LIT o TRAIL-R3) (Pan *et al.*, *Science*, 276:111-113 (1997); Sheridan *et al.*, *Science*, 277:818-821 (1997); McFarlane *et al.*, *J. Biol. Chem.*, 272:25417-25420 (1997); Schneider *et al.*, *FEBS Letters*, 416:329-334 (1997); Degli-Esposti *et al.*, *J. Exp. Med.*, 186:1165-1170 (1997); y Mongkolsapaya *et al.*, *J. Immunol.*, 160:3-6 (1998); DcR2 (llamado igualmente TRUNDD ó TRAIL-R4) (Marsters *et al.*, *Curr. Biol.*, 7:1003-1006 (1997); Pan *et al.*, *FEBS Letters*, 424:41-45 (1998); Degli-Esposti *et al.*, *Immunity*, 7:813-820 (1997), y OPG. En contraste con DR4 y DR5, los receptores DcR1 y DcR2 no señalizan apoptosis.

Algunos anticuerpos que se unen a los receptores DR4 y/o DR5 se han divulgado en la literatura. Por ejemplo, los anticuerpos anti-DR4 dirigidos al receptor DR4 y que poseen actividad agonística o apoptótica en ciertas células mamíferas se describen en, por ejemplo, WO 99/37684 publicada el 29 de julio de 1999, WO 00/73349 publicada el 12 de julio de 2000; WO 03/066661 publicada el 14 de agosto de 2003. Véase, además, por ejemplo, Griffith *et al.*, *J. Immunol.*, 162:2597-2605 (1999); Chuntharapai *et al.*, *J. Immunol.*, 166:4891-4898 (2001); WO 02/097033 publicada el 2 de diciembre de 2002; WO 03/042367 publicada el 22 de mayo de 2003; WO 03/038043 publicada el 8 de mayo de 2003; WO 03/037913 publicada el 8 de mayo de 2003. Igualmente, se han descrito

ciertos anticuerpos anti-DR5, véase, por ejemplo, WO 98/51793 publicada el 8 de noviembre de 1998; Griffith *et al., J. Immunol.*, 162:2597-2605 (1999); Ichikawa *et al., Nature Med.*, 7:954-960 (2001); Hylander *et al.*, "An Antibody to DR5 (TRAIL-Receptor 2) Suppresses the Growth of Patient Derived Gastrointestinal Tumors Grown in SCID mice", Abstract, 2d International Congress on Monoclonal Antibodies in Cancers, Aug. 29-Sept. 1, 2002, Banff, Alberta, Canada; WO 03/038043 publicada el 8 de mayo de 2003; WO 03/037913 publicada el 8 de mayo de 2003. Además, se han descrito ciertos anticuerpos con reactividad cruzada frente a los dos receptores, DR4 y DR5 (véase, por ejemplo, Patente de los Estados Unidos 6,252,050 expedida el 26 de junio de 2001).

Sumario de la Invención

La invención en esta solicitud se refiere, por lo menos en parte, a la identificación de acetato de histidina, pH 5.5 a 6.5, como un amortiguador particularmente útil para formular anticuerpos monoclonales, especialmente anticuerpos IgG1 de longitud total, los cuales son susceptibles a desamidación y/o agregación. La formulación retarda la degradación del producto anticuerpo en ella.

En consecuencia, en un primer aspecto, la invención concierne a una formulación farmacéutica estable, que comprende un anticuerpo monoclonal en amortiguador de acetato de histidina, pH 5.5 a 6.5. El anticuerpo monoclonal preferiblemente une un antígeno seleccionado del grupo conformado por HER2, CD20, DR5, BR3, IgE, y VEGF.

Adicionalmente, la invención concierne a un método de tratar una enfermedad o trastorno en un sujeto, que comprende administrar la formulación a un sujeto en una cantidad efectiva para tratar la enfermedad o trastorno.

En otro aspecto, la invención concierne a una formulación farmacéutica que comprende: (a) un anticuerpo IgG1 de longitud total susceptible a desamidación o agregación en una cantidad desde alrededor de 10mg/mL a alrededor de 250mg/mL; (b) amortiguador de acetato de

histidina, pH 5.5 a 6.5; (c) sacárido seleccionado del grupo conformado por trehalosa y sacarosa, en una cantidad desde alrededor de 60mM a alrededor de 250mM; y (d) polisorbato 20 en una cantidad desde alrededor de 0.01% a alrededor de 0.1%.

La invención provee asimismo un método para reducir la desamidación o agregación de un anticuerpo monoclonal terapéutico, que comprende formular el anticuerpo en un amortiguador de acetato de histidina, pH 5.5 a 6.5.

En todavía un aspecto adicional, la invención concierne a una formulación farmacéutica que comprende un anticuerpo que se une al dominio II de HER2 en un amortiguador de histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido y un surfactante.

La invención se refiere también a una formulación farmacéutica que comprende Pertuzumab en una cantidad desde alrededor de 20mg/mL a alrededor de 40mg/mL, amortiguador de acetato de histidina, sacarosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5.

La invención corresponde asimismo a una formulación farmacéutica que comprende un anticuerpo DR5 en un amortiguador de histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido, y un surfactante.

En otro aspecto, la invención concierne a una formulación farmacéutica que comprende Apomab en una cantidad desde alrededor de 10mg/mL a alrededor de 30mg/mL, amortiguador de acetato de histidina, trehalosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5.

En todavía otro aspecto, la invención provee un método de tratar cáncer en un sujeto, que comprende administrar la formulación farmacéutica al sujeto en una cantidad efectiva para tratar el cáncer.

La invención concierne a un frasquito con un tapón perforable por una jeringa o un recipiente de acero inoxidable, que comprende la formulación dentro del frasquito o recipiente, opcionalmente en forma congelada. Más aún, la invención provee un método de elaborar una

formulación farmacéutica que comprende: (a) preparar la formulación de anticuerpo monoclonal; y (b) evaluar la estabilidad física, la estabilidad química, o actividad biológica del anticuerpo monoclonal de la formulación.

Breve Descripción de los Dibujos

Figura 1 ilustra los Dominios I-IV (SEQ ID. Nos.19-22, respectivamente) del dominio extracelular de HER2.

Figuras 2A y 2B ilustran las alineaciones de las secuencias de aminoácidos de los dominios ligeros variables (V_L) (Fig. 2A) y pesados variables (V_H) (Fig. 2B) del anticuerpo monoclonal murino 2C4 (SEQ ID Nos. 1 y 2, respectivamente); dominios V_L y V_H de la versión 574 de 2C4 humanizado (SEQ ID Nos. 3 y 4, respectivamente), y las estructuras alternas (*"frameworks"*) de consenso V_L y V_H humanas (hum κ1, subgrupo I kappa ligero; humIII, subgrupo III pesado) (SEQ ID Nos. 5 y 6, respectivamente). Los asteriscos identifican diferencias entre la versión 574 de 2C4 humanizado y el anticuerpo monoclonal 2C4 murino o entre la versión 574 de 2C4 humanizado y la estructura alterna humana. Las Regiones Determinantes de Complementariedad (CDR) se encuentran entre paréntesis.

Figuras 3A y 3B muestran las secuencias de aminoácidos de la cadena ligera y la cadena pesada de Pertuzumab (SEQ ID Nos. 15 y 16, respectivamente). Las CDR se muestran en negrilla. La masa molecular calculada de la cadena ligera y la cadena pesada son 23.526,22 Da y 49.216,56 Da (cisternas en forma reducida). La fracción carbohidrato se une a Asn 299 de la cadena pesada.

Figuras 4A y 4B muestran las secuencias de aminoácidos de la cadena ligera y pesada de Pertuzumab, incluyendo cada una secuencia peptídica señal aminoterminal intacta (SEQ ID Nos. 17 y 18, respectivamente).

Figura 5 ilustra, esquemáticamente, la unión de 2C4 en el sitio heterodimérico de unión de HER2, previniendo en esa forma la heterodimerización con EGFR o HER3 activados.

Figura 6 ilustra el acoplamiento de HER2/HER3 a las rutas MAPK y Akt.

Figura 7 compara las actividades de Trastuzumab y Pertuzumab.

Figura 8 ilustra la estabilidad de la formulación de Pertuzumab mediante análisis de intercambio iónico (IEX).

Figura 9 muestra la estabilidad de la formulación de Pertuzumab mediante análisis de cromatografía de exclusión de tamaño (SEC).

Figura 10 refleja la estabilidad física de Pertuzumab en diferentes formulaciones.

Figura 11 es de un estudio de agitación de formulaciones líquidas de Pertuzumab.

Figura 12 es de otro estudio de agitación de formulaciones líquidas de Pertuzumab.

Figura 13 es de un estudio de congelamiento-descongelamiento de una formulación de Pertuzumab.

Figuras 14A y 14B muestran las secuencias de aminoácidos de la cadena ligera (SEQ ID No. 13) y la cadena pesada (SEQ ID No. 14) de Trastuzumab.

Figuras 15A y 15B ilustran una secuencia variante de cadena ligera (SEQ ID No. 23) de Pertuzumab y una secuencia variante de cadena pesada (SEQ ID No. 24) de Pertuzumab.

Figura 16A y 16B muestra estructuras de oligosacáridos observadas comúnmente en anticuerpos IgG.

Figuras 17A y 17B muestran las secuencias de las cadenas ligeras y pesadas (SEQ ID Nos. 37-44) de anticuerpos anti-IgE específicos E25, E26, HAE1 y Hu-901. En Fig. 17A, el dominio ligero variable termina con los residuos VEIK, alrededor del residuo 111. En Fig. 17B, el dominio pesado variable termina con los residuos VTVSS, alrededor del residuo 120.

Figura 18A es una alineación de secuencias que compara las secuencias de aminoácidos del dominio ligero variable (V_L) de cada uno de 2H7 murino (SEQ ID No. 25), la variante 2H7v16 humanizada (SEQ ID No. 26), y el subgrupo I de cadena ligera kappa humano (SEQ ID No. 27). Las CDR de V_L de 2H7 y hu2H7vl6 son las siguientes: CDRl (SEQ ID No. 57), CDR2 (SEQ ID No. 58), y CDR3 (SEQ ID No. 59).

Figura 18B es una alineación de secuencias que compara las secuencias de aminoácidos del dominio pesado variable (V_H) de cada uno de 2H7 murino (SEQ ID No. 28), la variante 2H7v16 humanizada (SEQ ID No. 29), y la secuencia consenso humana del subgrupo III de cadena pesada (SEQ ID No. 30). Las CDR de V_H de 2H7 y hu2H7v16 son las siguientes: CDRl (SEQ ID No. 60), CDR2 (SEQ ID No. 61), y CDR3 (SEQ ID No. 62).

En Fig. 18A y Fig. 18B, las CDRl, CDR2 y CDR3 en cada cadena se encierran entre paréntesis, flanqueadas por las regiones de estructura alterna de anticuerpo, FR1-FR4, como se indica. 2H7 se refiere al anticuerpo 2H7 murino. Los asteriscos entre dos filas de secuencias indican las posiciones que son diferentes entre las dos secuencias. La numeración de los residuos es de acuerdo con Kabat *et al.* Sequences of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), con inserciones ilustradas como a, b, c, d, y e.

Figura 19 ilustra secuencias de dominio variable de tres diferentes anticuerpos VEGF con SEQ ID Nos. 31- 36.

Figura 20 muestra el perfil de elución por cromatografía de exclusión de tamaño de las siguientes muestras Apomab: (a) control y formulaciones preparadas con (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 y (e) pH 7.0. Las muestras formuladas se almacenaron a 40°C durante 2 meses antes del análisis.

Figura 21 ilustra el perfil de la tasa de pH para la pérdida en monómero del anticuerpo Apomab durante el almacenamiento. La cinética del monómero mediante SEC se monitoreó durante el almacenamiento a 30°C y 40°C, y se calcularon las constantes de tasa de primer orden.

Figura 22 presenta el perfil de elución de cromatografía de intercambio iónico (IEC) de muestras de Apomab en la siguiente forma: (a) control y formulaciones preparadas con (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 y (e) pH 7.0. Las muestras formuladas se almacenaron a 40°C durante 2 meses antes del análisis.

Figura 23 muestra el perfil de tasa de pH para la pérdida en el pico principal de IEC durante el almacenamiento. La cinética de pico principal por IEC se monitoreó durante el almacenamiento a 30°C y 40°C, y se calcularon las constantes de tasa de primer orden.

Figura 24 muestra la secuencia de nucleótidos del ADNc del ligando Apo-2 humano (SEQ ID No. 45) y su secuencia derivada de aminoácidos (SEQ ID No. 46). La "N" en la posición de nucleótido 447 (en SEQ ID No. 45) se usa para indicar que la base del nucleótido puede ser una "T" o "G".

Figuras 25 A y 25B muestran la secuencia de 411 aminoácidos del receptor DR5 humano (SEQ ID No.47) como se publica en WO 98/51793 el 19 de noviembre de 1998, y la secuencia nucleotídica codificante (SEQ ID No. 48).

Figuras 26A y 26B muestran la secuencia de 440 aminoácidos del receptor DR5 humano (SEQ ID No. 49) y la secuencia nucleótídica codificante (SEQ ID No. 50), como también se publica en WO 98/35986 el 20 de agosto de 1998.

Figura 27 es la secuencia de aminoácidos de cadena pesada de Apomab 7.3 (SEQ ID No. 51).

Figura 28 es la secuencia de aminoácidos de cadena ligera de Apomab 7.3 (SEQ ID No.52).

Figuras 29 muestran la alineación de la cadena pesada 16E2 (SEQ ID No. 53) y las secuencias de aminoácidos de cadena pesada 7.3 (SEQ ID No. 51) de Apomab.

Figura 30 muestra la alineación las secuencias de aminoácidos de la cadena ligera 16E2 (SEQ ID No. 54) y la cadena ligera de Apomab 7.3 (SEQ ID No. 52).

Figuras 31A y 31B ilustran la secuencia de aminoácidos pesada variable (Fig. 31A; SEQ ID No. 55) y la secuencia de aminoácidos ligera variable (Fig. 31B; SEQ ID No. 56) de Apomab 7.3. Los residuos de CDR se identifican en negrilla.

Figura 32 muestra una alineación de las cadenas ligeras de 2H7vl6 y 2H7v511 maduras (SEQ ID Nos. 63 y 64, respectivamente). Las secuencias se muestran con la numeración de residuos de dominio variable de Kabat y la numeración de residuos de dominio constante Eu.

Figura 33 muestra una alineación de las cadenas pesadas maduras 2H7vl6 y 2H7v511 (SEQ ID Nos. 65 y 66, respectivamente). Las secuencias se muestran con la numeración de residuos de dominio variable de Kabat y la numeración de residuos de dominio constante Eu.

Descripción Detallada de las Incorporaciones Preferidas

I. Definiciones

El término "formulación farmacéutica" se refiere a una preparación que está en tal forma como para permitir que la actividad biológica del ingrediente activo sea efectiva, y que no contiene componentes adicionales que sean inaceptablemente tóxicos a un sujeto, al cual se administraría la formulación. Tales formulaciones son estériles.

Una "formulación estéril" es aséptica o libre de todos los microorganismos vivos y sus esporas.

En esta solicitud, una formulación "congelada" es una formulación a una temperatura por debajo de 0° C. Generalmente, la formulación congelada no se seca por congelamiento, ni se somete a liofilización previa o subsiguiente. Preferiblemente, la formulación congelada comprende una sustancia farmacéutica congelada para almacenamiento (en un recipiente de acero inoxidable) o un producto farmacéutico congelado (en configuración de frasquito final).

Una la formulación "estable" es una en la cual la proteína contenida en ella conserva esencialmente su estabilidad física y/o estabilidad química y/o actividad biológica luego del almacenamiento. Preferiblemente, la

formulación conserva esencialmente su estabilidad física y química, así como su actividad biológica luego del almacenamiento. El período de almacenamiento se selecciona generalmente basándose en la semivida prevista de la formulación. En el arte se dispone de varias técnicas analíticas para medir la estabilidad de una proteína y se reseñan en Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993), por ejemplo. La estabilidad puede medirse a una temperatura seleccionada durante un período de tiempo elegido. Preferiblemente, la formulación es estable a alrededor de 40°C durante por lo menos alrededor de 2-4 semanas, y/o estable a alrededor de 5°C y/o 15°C durante por lo menos 3 meses, y/o estable a alrededor de -20°C durante por lo menos 3 meses o por lo menos 1 año. Más aún, la formulación es preferiblemente estable luego del congelamiento (a, por ejemplo, -70°C) y descongelamiento de la formulación, por ejemplo luego de 1, 2 ó 3 ciclos de congelamiento y descongelamiento. La estabilidad puede evaluarse cualitativa y/o cuantitativamente en una variedad de diferentes modos, incluida la evaluación de la formación de agregados (por ejemplo usando cromatografía de exclusión de tamaño, mediante la turbiedad, y/o mediante inspección visual); evaluando la heterogeneidad de carga utilizando cromatografía de intercambio catiónico o eletroforesis de zona capilar; análisis de secuencias amino-terminales o carboxi- terminales; análisis espectrométrico de masas; análisis SDS-PAGE para comparar anticuerpo reducido e intacto; análisis de mapa peptídico (por ejemplo tríptico o LYS-C); evaluando la actividad biológica o la función de unión a antígeno del anticuerpo; etc. La inestabilidad puede involucrar una cualquiera o más de: agregación, desamidación (por ejemplo, desamidación Asn), oxidación (por ejemplo, oxidación Met), isomerización (por ejemplo, isomerización Asp), corte/hidrólisis/fragmentación (por ejemplo, fragmentación de unión flexible), formación se succinimida, cisteína(s) no pareada, extensión N-terminal, procesamiento C-terminal, diferencias de glucosilación, etc. En esta solicitud, un anticuerpo monoclonal "desamidado" es uno en el cual

uno o más residuos asparagina del mismo se han derivado, por ejemplo, a un ácido aspártico o a un ácido iso-aspártico.

Un anticuerpo que es "susceptible a desamidación" es uno que comprende uno o más residuos, que se ha descubierto que son propensos a desamidarse.

Un anticuerpo que es "susceptible a agregación" es uno que se ha descubierto que se agrega con otra molécula(s) de anticuerpo, especialmente luego del congelamiento y/o agitación.

Un anticuerpo que es "susceptible a fragmentación" es uno que se ha descubierto que se corta proteolíticamente en dos o más fragmentos, por ejemplo en una región de unión flexible del mismo.

Por "desamidación reductora, agregación, o fragmentación" se quiere dar a entender prevenir o disminuir la cantidad de desamidación, agregación, o fragmentación relativa al anticuerpo monoclonal formulado con un pH diferente o en un amortiguador diferente.

En esta solicitud, "actividad biológica" de un anticuerpo monoclonal se refiere a la capacidad del anticuerpo de unirse a antígeno y resultar en una respuesta biológica cuantificable, la cual puede medirse *in vitro* o *in vivo*. Tal actividad puede ser antagonística (por ejemplo donde el anticuerpo es un anticuerpo HER2) o agonística (por ejemplo, donde el anticuerpo une DR5). En el caso de Pertuzumab, en una incorporación, la actividad biológica se refiere a la capacidad del anticuerpo formulado de inhibir la proliferación de la línea celular MDA-MB-175-VII de cáncer de mama humano. Donde el anticuerpo es Apomab, la actividad biológica puede referirse, por ejemplo, a la capacidad del anticuerpo formulado de eliminar las células Colo205 del carcinoma del colon.

Por "isotónica" se quiere significar que la formulación de interés tiene esencialmente la misma presión osmótica que la sangre humana. Las formulaciones isotónicas tendrán generalmente una presión osmótica desde alrededor de 250 a 350mOsm. La isotonicidad puede medirse usando un osmómetro tipo presión de vapor o congelamiento con hielo, por ejemplo.

Tal como se emplea en esta solicitud, "amortiguador" se refiere a una solución amortiguada que resiste cambios en el pH por la acción de sus componentes conjugados ácido-base. El amortiguador de esta invención tiene preferiblemente un pH en el rango desde alrededor de 5.0 a alrededor de 7.0, preferiblemente desde alrededor de 5.5 a alrededor de 6.5, por ejemplo desde alrededor de 5.8 a alrededor de 6.2 y, muy preferiblemente, tiene un pH de alrededor de 6.0. Ejemplos de amortiguadores que controlarán el pH en este rango incluyen acetato, succinato, gluconato, histidina, citrato, glicilglicina y otros amortiguadores ácidos orgánicos. El amortiguador preferido en esta solicitud es un amortiguador de histidina.

Un "amortiguador de histidina" es un amortiguador que comprende iones de histidina. Ejemplos de amortiguadores de histidina incluyen cloruro de histidina, acetato de histidina, fosfato de histidina, sulfato de histidina. Se descubrió que el amortiguador de histidina preferido identificado en los ejemplos en esta solicitud es el acetato de histidina. En la incorporación preferida, el amortiguador de acetato de histidina se prepara valorando volumétricamente L-histidina (base libre, sólido) con ácido acético (líquido). Preferiblemente, el amortiguador de histidina o amortiguador de acetato de histidina está en un pH 5.5 a 6.5, preferiblemente pH 5.8 a 6.2.

Un "sacárido" en esta solicitud comprende la composición general (CH2O)n y sus derivados, incluidos monosacáridos, disacáridos, trisacáridos, polisacáridos, alcoholes de azúcar, azúcares reductores, azúcares no reductores, etc. Ejemplos de sacáridos en esta solicitud incluyen glucosa, sacarosa, trehalosa, lactosa, fructosa, maltosa, dextrina, glicerina, dextrano, eritritol, glicerol, arabitol, silitol, sorbitol, mannitol, melibiosa, melezitosa, rafinosa, mannotriosa, estaquiosa, maltosa, lactulosa, maltulosa, glucitol, maltitol, lactitol, iso-maltulosa, etc. El sacárido preferido en esta solicitud es un disacárido no reductor, tal como trehalosa o sacarosa.

En esta solicitud, un "surfactante" se refiere a un agente tenso-activo, preferiblemente un surfactante no iónico. Ejemplos de surfactantes en esta

solicitud incluyen polisorbato (por ejemplo, polisorbato 20 y polisorbato 80); poloxámero (por ejemplo, poloxámero 188); Triton; dodecilsulfato de sodio (SDS); laurel sulfato de sodio; octil-glucósido de sodio; lauril-, miristil-, linoleil-, o estearil-sulfobetaína; lauril-, miristil-, linoleil- o estearil-sarcosina; linoleil-, miristil-, o cetil-betaína; lauroamidopropil-, cocamidopropil-, linoleamidopropil-, miristamidopropil-, palmidopropil-, o isostearamidopropil-betaína (por ejemplo lauroamidopropil); miristamidopropil-, palmidopropil-, o isostearamidopropil-dimetilamina; metil cocoiltaurato sódico, o metil oleil-taurato disódico; y la serie MONAQUAT™ (Mona Industries, Inc., Paterson, New Jersey); polietil glicol, polipropil glicol, y copolímeros de etilen y propilen glicol (por ejemplo, Pluronics, PF68 etc); etc. El surfactante preferido en esta solicitud es polisorbato 20.

Un "receptor HER" es una proteína tirosina cinasa de receptor que pertenece a la familia de receptores HER e incluye los receptores EGFR, HER2, HER3 y HER4 y otros miembros de esta familia a identificarse en el futuro. El receptor HER comprenderá generalmente un dominio extracelular, que puede unir un ligando HER; un dominio lipófilo de transmembrana; un dominio tirosina cinasa intracelular conservado; y un dominio de señalización carboxilo-terminal que alberga varios residuos de tirosina, los cuales pueden fosforilarse. Preferiblemente el receptor HER une el receptor HER humano de secuencia natural.

El dominio extracelular de HER2 comprende cuatro dominios, Dominio I (residuos de aminoácidos desde alrededor de 1-195), Dominio II (residuos de aminoácidos desde alrededor de 196-320), Dominio III (residuos de aminoácidos desde alrededor de 321- 488), y Dominio IV (residuos de aminoácidos desde alrededor de 489-632) (numeración de residuos sin péptido señal). Véase Garrett *et al., Mol. Cell.*, 11: 495-505 (2003), Cho *et al., Nature* 421 : 756-760 (2003), Franklin *et al., Cancer Cell* 5:317-328 (2004), o Plowman *et al., Proc. Natl. Acad. Sci.* 90:1746-1750 (1993). Véase también Fig. 1 en esta solicitud.

Los términos "ErbB1", "HER1", "receptor de factor de crecimiento epidérmico" y "EGFR" se emplean indistintamente en esta solicitud y se refieren a EGFR como se divulga, por ejemplo, en Carpenter *et al., Ann. Rev. Biochem.* 56:881-914 (1987), incluidas sus formas mutantes de ocurrencia natural (*por ejemplo*, un EGFR mutante de deleción como en Humphrey *et al., PNAS (USA)* 87:4207-4211 (1990)). *erb*B1 se refiere al gen que codifica el producto proteína EGFR.

Las expresiones "ErbB2" y "HER2" se emplean indistintamente en esta solicitud y se refieren a la proteína HER2 humana, descrita por ejemplo en Semba *et al., PNAS (USA)*, 82:6497-6501 (1985) y Yamamoto *et al., Nature*, 319:230-234 (1986) (número de acceso a Genbank X03363). El término "*erb*B2" se refiere al gen que codifica el ErbB2 humano y "*neu*" se refiere al gen que codifica p185^{neu} de rata. El HER2 preferido es HER2 humano de secuencia natural.

"ErbB3" y "HER3" se refieren al polipéptido receptor como se divulga, por ejemplo, en las Patentes de los Estados Unidos No. 5,183,884 y 5,480,968, así como Kraus *et al., PNAS (USA)* 86:9193-9197 (1989).

Los términos "ErbB4" y "HER4" en esta solicitud se refieren al polipéptido 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, publicada el 22 de abril de 1999.

Por "ligando HER" se da a entender un polipéptido que se une a un receptor HER y/o lo activa. El ligando HER de particular interés en esta solicitud es un ligando HER humano de secuencia natural tal como el factor de crecimiento epidérmico (EGF) (Savage *et al., J. Biol. Chem.* 247:1612-1621 (1972)); factor α de crecimiento transformante (TGF-α) (Marquardt *et al., Science* 223:1079-1082 (1984)); anfirregulina también conocida como schwanoma o factor de crecimiento autocrino de queratinocitos (Shoyab *et al., Science* 243: 1074-1076 (1989); Kimura *et al., Nature* 348:257-260

(1990); y Cook *et al., Mol. Cell. Biol.* 11:2547-2557 (1991)); betacelulina (Shing *et al., Science* 259:1604-1607 (1993); y Sasada *et al., Biochem. Biophys. Res. Commun.* 190: 1173 (1993)); factor de crecimiento epidérmico de unión a heparina (HB-EGF) (Higashiyama *et al., Science* 251:936-939 (1991)); epirregulina (Toyoda *et al., J. Biol. Chem.* 270:7495-7500 (1995); y Komurasaki *et al., Oncogene* 15:2841-2848 (1997)); una herregulina (véase más adelante); neurregulina-2 (NRG-2) (Carraway *et al., Nature* 387:512-516 (1997)); neurregulina-3 (NRG-3) (Zhang *et al., Proc. Natl. Acad. Sci.* 94:9562-9567 (1997)); neurregulina-4 (NRG-4) (Harari *et al., Oncogene* 18:2681-89 (1999)) o cripto (CR-1) (Kannan *et al., J. Biol. Chem.* 272(6):3330-3335 (1997)). Los ligandos HER que unen EGFR incluyen EGF, TGF-α, anfirregulina, betacelulina, HB-EGF y epirregulina. Los ligandos HER que unen HER3 incluyen herregulinas. Ligandos HER capaces de unir HER4 incluyen betacelulina, epirregulina, HB-EGF, NRG-2, NRG-3, NRG-4 y herregulinas.

"Herregulina" (HRG), cuando se emplea en esta solicitud, se refiere a un polipéptido codificado por el producto génico herregulina como se divulga en la Patente de los Estados Unidos No. 5,641,869 o Marchionni *et al., Nature* 362:312-318 (1993). Ejemplos de herregulinas incluyen herregulina-α, herregulina-β1, herregulina-β2 y herregulina-β3 (Holmes *et al., Science* 256:1205-1210 (1992); y Patente de los Estados Unidos No.5,641,869); factor de diferenciación *neu* (NDF) (Peles *et al., Cell* 69: 205-216 (1992)); actividad inductora de receptor acetilcolina (ARIA) (Falls *et al., Cell* 72:801-815 (1993)); factores de crecimiento glial (GGFs) (Marchionni *et al., Nature*, 362:312-318 (1993)); factor derivado de neuronas motoras y sensoriales (SMDF) (Ho *et al., J. Biol. Chem.* 270:14523-14532 (1995)); γ-herregulina (Schaefer *et al., Oncogene*, 15:1385-1394 (1997)). El término incluye fragmentos biológicamente activos y variantes de secuencia de aminoácidos de un polipéptido HRG de secuencia natural, tal como su fragmento de dominio tipo EGF (*por ejemplo*, $HRG\beta1_{177-244}$).

Un "dímero HER" en esta solicitud es un dímero no covalentemente asociado, que comprende por lo menos dos diferentes receptores HER. Tales complejos pueden formarse cuando una célula, que expresa dos o más receptores HER, se expone a un ligando HER y puede aislarse por inmunoprecipitación y analizarse por SDS-PAGE como se describe en Sliwkowski *et al., J. Biol. Chem.*, 269(20):14661-14665 (1994), por ejemplo. Ejemplos de tales dímeros HER incluyen los heterodímeros EGFR-HER2, HER2-HER3 y HER3-HER4. Más aún, el dímero HER puede comprender dos o más receptores HER2, combinados con un receptor HER diferente, tal como HER3, HER4 o EGFR. Otras proteínas, tales como la subunidad de receptor de citosina (por ejemplo, gp130) pueden asociarse con el dímero.

Un "sitio heterodimérico de unión" en HER2, se refiere a una región en el dominio extracelular de HER2 que contacta, o interactúa con, una región en el dominio extracelular de EGFR, HER3 o HER4, luego de la formación de un dímero con ella. La región se encuentra en Dominio II de HER2, Franklin *et al., Cancer Cell* 5:317-328 (2004).

"Activación de HER" o "activación de HER2" se refiere a la activación, o fosforilación, de uno cualquiera o más receptores HER, o receptores HER2. Generalmente, la activación de HER resulta en transducción de señales (por ejemplo, la causada por un dominio cinasa intracelular de un receptor HER, que fosforila residuos de tirosina en el receptor HER o un polipéptido sustrato). La activación de HER puede estar mediada por la unión de ligando HER a un dímero HER, que comprende el receptor HER de interés. La unión de ligando HER a un dímero HER puede activar un dominio cinasa de uno o más de los receptores HER en el dímero y, por consiguiente, resulta en la fosforilación de residuos de tirosina en uno o más de los receptores HER y/o fosforilación de residuos de tirosina en polipéptido(s) sustrato(s) adicional(es), tales como cinasas intracelulares Akt o MAPK.

El término "anticuerpo" en esta solicitud se emplea en el sentido más amplio y cubre específicamente anticuerpos monoclonales de longitud total,

anticuerpos policlonales, anticuerpos multiespecíficos (*por ejemplo*, anticuerpos biespecíficos) formados, por lo menos, de dos anticuerpos de longitud total, y fragmentos de anticuerpo, siempre y cuando exhiban la actividad biológica deseada.

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 y/o unen el mismo epítopo, excepto por posibles variantes que pueden surgir durante la producción del anticuerpo monoclonal, estando tales variantes generalmente presentes en cantidades menores. En contraste con preparaciones de anticuerpo policlonal que, típicamente, 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 otras inmunoglobulinas. 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.*, *Nature*, 256: 495 (1975), o pueden elaborarse por métodos de ADN recombinante (véase, *por ejemplo*, Patente de los Estados Unidos 4,816,567). Los "anticuerpos monoclonales" pueden aislarse también de bibliotecas de anticuerpos en bacteriófagos, usando las técnicas descritas en Clackson *et al.*, *Nature*, 352:624-628 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), 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 (Patente de los Estados Unidos 4,816,567; y Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855(1984)). Los anticuerpos quiméricos de interés en esta solicitud incluyen anticuerpos "primatizados", que comprenden secuencias de unión a antígeno de dominio variable, derivados de un primate no humano (*por ejemplo*, mono del Viejo Mundo o macaco) y secuencias de región constante humanas.

"Fragmentos de anticuerpo" comprenden una porción de un anticuerpo de cadena total, que contiene preferiblemente su región variable o de unión a antígeno. Ejemplos de fragmentos de anticuerpo incluyen fragmentos Fab, Fab', $F(ab')_2$ y Fv; diacuerpos; anticuerpos lineales; moléculas de anticuerpo de cadena única; y anticuerpos multiespecíficos formados de fragmento(s) de anticuerpo.

Un "anticuerpo de longitud total" es uno que comprende una región variable de unión a antígeno, así como un dominio constante de cadena ligera (C_L) y dominios constantes de cadena pesada, C_H1, C_H2 y C_H3. Los dominios constantes pueden ser dominios constantes de secuencia natural (por ejemplo, dominios constantes de secuencia natural humana) o sus variantes de secuencia de aminoácidos. Preferiblemente, el anticuerpo de longitud total tiene una o más funciones efectoras.

El término "anticuerpo de especie principal" en esta solicitud se refiere a una estructura de anticuerpo en una composición, que es la molécula de anticuerpo cuantitativamente predominante en la composición. En una incorporación, el anticuerpo de especie principal es un anticuerpo HER2, tal como un anticuerpo que se une al Dominio II de HER2, anticuerpo que inhibe la dimerización de HER más efectivamente que Trastuzumab, y/o un anticuerpo que se une a un sitio heterodimérico de unión de HER2. La incorporación preferida en esta solicitud de un

anticuerpo HER2 de especie principal es uno que comprende las secuencias de aminoácidos variables ligeras y pesadas variables en SEQ ID Nos. 3 y 4 y, muy preferiblemente que comprende las secuencias de aminoácidos de cadena ligera y cadena pesada en SEQ ID Nos. 15 y 16 (Pertuzumab).

Una "variante de secuencia de aminoácidos" en esta solicitud es un anticuerpo con una secuencia de aminoácidos que difiere de un anticuerpo de especie principal. Ordinariamente, las variantes de secuencia de aminoácidos poseerán por lo menos alrededor de 70% de homología con el anticuerpo de especie principal y, preferiblemente, serán por lo menos alrededor de 80%, más preferiblemente por lo menos alrededor de 90% homólogas con el anticuerpo de especie principal. Las variantes de secuencia de aminoácidos poseen sustituciones, deleciones, y/o adiciones en ciertas posiciones en, o adyacentes, a la secuencia de aminoácidos del anticuerpo de especie principal. Ejemplos de variantes de secuencia de aminoácidos en esta solicitud incluyen variante ácida (por ejemplo, variante de anticuerpo desamidado), variante básica, el anticuerpo con una extensión líder amino-terminal (por ejemplo, VHS-) en una o dos de sus cadenas ligeras, anticuerpo con un residuo lisina C-terminal en una o dos de sus cadenas pesadas, etc, e incluye combinaciones de variaciones a las secuencias de aminoácidos de cadenas pesadas y/o ligeras. La variante del anticuerpo de particular interés en esta solicitud es el anticuerpo que comprende una extensión líder amino-terminal en una o más de sus cadenas ligeras, comprendiendo además, en forma opcional, otras diferencias de secuencia de aminoácidos y/o glucosilación, relativas al anticuerpo de especie principal.

Un "anticuerpo monoclonal terapéutico" es un anticuerpo usado para terapia de un sujeto humano. Anticuerpos monoclonales terapéuticos divulgados en esta solicitud incluyen: anticuerpos HER2 para el cáncer y varias enfermedades o trastornos no malignos; anticuerpos CD20 o BR3 para terapias de neoplasias malignas de linfocitos B, enfermedades autoinmunitarias, rechazo de injertos, o bloqueo de una respuesta inmunitaria a un antígeno extraño; anticuerpos IgE para terapia de un

trastorno mediado por IgE; anticuerpos DR5 o VEGF para terapia contra el cáncer.

Un anticuerpo de "variante de glucosilación" en esta solicitud es un anticuerpo con una o más fracciones carbohidrato unidas a él, que difieren de una o más fracciones carbohidrato unidas a un anticuerpo de especie principal. Ejemplos de variantes de glucosilación en esta solicitud incluyen un anticuerpo con una estructura oligosacárido Gl o G2, en vez de una estructura de oligosacárido GO, unida a una región Fc del mismo, un anticuerpo con una o más fracciones carbohidrato unidas a una o dos cadenas ligeras del mismo, un anticuerpo sin un carbohidrato unido a una o dos cadenas pesadas del anticuerpo, etc, y combinaciones de alteraciones de glicosilación.

Donde el anticuerpo tiene una región Fc, una estructura oligosacárido, tal como la mostrada en Fig. 16 en esta solicitud, puede unirse a una o dos cadenas pesadas del anticuerpo, por ejemplo, en el residuo 299 (298, numeración Eu de residuos). Para Pertuzumab, GO fue la estructura oligosacárido predominante, encontrándose otras estructuras oligosacárido tales como GO-F, G-1, Man5, Man6, G1-1, G1(1-6), G1(1-3) y G2 en cantidades menores en la composición Pertuzumab.

A menos que se indique lo contrario, una "estructura oligosacárido Gl" en esta solicitud incluye las estructuras G-1, G1-1, G1(1-6) y G1(1-3).

Una "extensión líder amino-terminal" en esta solicitud se refiere a uno o más residuos de aminoácidos de la secuencia líder amino-terminal, que están presentes en el término amino de una cualquiera o más cadenas pesadas o ligeras de un anticuerpo. Una extensión líder amino-terminal ejemplar comprende o consta de tres residuos de aminoácidos, VHS, presentes en una o ambas cadenas ligeras de una variante de anticuerpo.

"Homología" 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 (*"gaps"*), si es necesario, para lograr la homología porcentual máxima. Métodos y programas de computador para la alineación son bien conocidos en el arte. Un programa similar de

computador es "Align 2," creado por Genentech, Inc., y radicado con documentación de usuario ante la Oficina de los Estados Unidos de Derechos de Autor, Washington, DC 20559, el 10 de diciembre de 1991.

"Funciones efectoras" de anticuerpo se refieren a aquellas actividades biológicas atribuibles a la región Fc (una región Fc de secuencia natural o 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; regulación por disminución de receptores de superficie celular (por ejemplo, receptor de linfocitos B; BCR), etc.

Dependiendo de la secuencia de aminoácidos del dominio constante de sus cadenas pesadas, anticuerpos de longitud total pueden asignarse a diferentes "clases". Existen cinco clases principales de anticuerpos de longitud total: IgA, IgD, IgE, IgG, e IgM, y varias de éstas pueden subdividirse aún más 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. Son bien conocidas las estructuras subunitarias y configuraciones tridimensionales de diferentes clases de inmunoglobulinas.

"Citotoxicidad celular dependiente de anticuerpo" y "ADCC" se refieren a una reacción mediada por células, en la cual células citotóxicas no específicas, que expresan receptores Fc (FcR) (por ejemplo, linfocitos citolíticos naturales (NK), neutrófilos, y macrófagos), reconocen el anticuerpo unido en una célula diana y, posteriormente, causan lisis de la célula diana. Las células primarias para mediar ADCC, los linfocitos NK, expresan únicamente FcγRIII, mientras que los monocitos expresan FcγRI, FcγRII, y FcγRIII. La expresión de FcR en células hematopoyéticas se resume en la Tabla 3 en la página 464 de Ravetch *et al.*, *Annu. Rev. Immunol* 9:457-92 (1991). Para evaluar la actividad ADCC de una molécula de interés, puede realizarse un ensayo ADCC *in vitro*, tal como el descrito en la Patente de los Estados Unidos No. 5,500,362 o 5,821,337.

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 animal tal como el divulgado en Clynes *et al.*, *PNAS (USA)* 95:652-656 (1998).

"Células efectoras humanas" son leucocitos que expresan uno o más FcR y realizan funciones efectoras. Preferiblemente, las células expresan por lo menos FcγRIII y realizan la función efectora ADCC. Ejemplos de leucocitos humanos, que median ADCC, incluyen células moonucleares de sangre periférica (PBMC), linfocitos citolíticos naturales (NK), monocitos, linfocitos T citotóxicos y neutrófilos, prefiriéndose PBMC y linfocitos citolíticos naturales (NK). Las células efectoras pueden aislarse de una fuente natural de las mismas, por ejemplo, de sangre o PBMC como se describe en esta solicitud.

Los términos "receptor Fc" o "FcR" se usan para describir un receptor que se une a la región Fc de un anticuerpo. El FcR preferido es un FcR humano de secuencia natural. Más aún, un FcR preferido es uno que une un anticuerpo IgG (un receptor gamma) e incluye receptores de las subclases FcγRI, FcγRII, y FcγRIII, incluidas sus variantes alélicas y, alternativamente, formas empalmadas de estos receptores. Los receptores FcγRII incluyen FcγRIIA (un "receptor activador") y FcγRIIB (un "receptor inhibidor"), los cuales poseen secuencias de aminoácidos similares que difieren principalmente en sus dominios citoplásmicos. El receptor activador FcγRIIA contiene un motivo de activación a base de tirosina de inmunorreceptor (ITAM) en su dominio citoplásmico. El receptor inhibidor FcγRIIB contiene un motivo de inhibición a base de tirosina de inmunorreceptor (ITIM) en su dominio citoplásmico, (véase Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997). Los FcR se reseñan en Ravetch y Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et. al.*, *Immunomethods* 4:25-34 (1994); y de Haas *et. al.*, *J. Lab. Clin. Med.* 126: 330-41 (1995). Otros FcR, incluidos aquellos por identificar en el futuro, se abarcan por el término "FcR" contenido aquí. El término comprende asimismo el receptor

neonatal, FcRn, que es responsable de la transferencia de IgG maternas al feto. Guyer *et. al., J. Immunol.* 117: 587 (1976) y Kim *et. al., J. Immunol* 24:249 (1994).

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

"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 enlaza a una cadena pesada por un enlace disulfuro covalente, mientras que el número de enlazamientos disulfuro varía entre las cadenas pesadas de diferentes isotipos de inmunoglobulina. Cada cadena ligera y pesada posee también puentes disulfuro intracadena, regularmente espaciados. Cada cadena pesada tiene en un extremo un dominio variable (V_H), seguido por un número de dominios constantes. Cada cadena ligera tiene un dominio variable en un extremo (V_L) y un dominio constante en su 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 la 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 través 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 de estructura alterna (FR) (***"the framework regions (FRs)"***). Los dominios variables de cadenas naturales 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 a antígeno de anticuerpos (véase Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). 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 citotoxicidad celular, dependiente de anticuerpo (ADCC).

El término "región hipervariable", cuando se emplea en esta solicitud, se refiere a los residuos de aminoácidos de un anticuerpo que son responsables de la unión a antígeno. La región hipervariable comprende generalmente residuos de aminoácidos de una "región determinante de complementariedad" 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., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) 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 *J. Mol. Biol.* 196:901-917 (1987)). Residuos de "región de estructura alterna" o "FR" son aquellos residuos de dominio variable

distintos de los residuos de región hipervariable, como se definen en esta solicitud.

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

"Fv" es el fragmento de anticuerpo mínimo, que contiene un sitio completo de unión a antígeno y reconocimiento de antígeno. Esta región consta de un dímero de un dominio variable de cadena pesada y cadena ligera en asociación estrecha, no covalente. Es en esta configuración que las tres regiones hipervariables de cada dominio variable interactúan para definir un sitio de unión a antígeno en la superficie del dímero V_H-V_L. Colectivamente, las seis regiones hipervariables confieren especificidad de unión a 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 unir antígeno, si bien con una afinidad más baja que todo el sitio de unión.

El fragmento Fab contiene igualmente 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 pocos residuos en el término carboxi del dominio CH1 de cadena pesada, incluyendo una o más cisteínas de la región de unión 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 lleva por lo menos un grupo tiol libre. Fragmentos $F(ab')_2$ de anticuerpo se produjeron originalmente como pares de fragmentos Fab', que tienen cisteínas de unión flexible entre ellos. Otros acoplamientos químicos de fragmentos de anticuerpo son también conocidos.

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.

Fragmentos de anticuerpo "Fv de cadena única" o "scFv" comprenden los dominios V_H y V_L de anticuerpo, en donde estos dominios están presentes en una sola cadena polipeptídica. Preferiblemente, el polipéptido Fv comprende además un conector polipeptídico entre los dominios V_H y V_L, que permite que el scFv forme la estructura deseada para unión a antígeno. Para una reseña del scFv, véase Plückthun en *The Pharmacology of Monoclonal Antibodies,* vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, páginas 269-315(1994). Los fragmentos scFv del anticuerpo HER2 se describen en WO93/16185; Patente de los Estados Unidos No. 5,571,894; y Patente de los Estados Unidos No. 5,587,458.

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

Formas "humanizadas" de anticuerpos no humanos (por ejemplo, de 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 recipiendario), en las cuales residuos de una región hipervariable del recipiendario 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 de estructura alterna (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 recipiendario ni en anticuerpo donante. Estas modificaciones se hacen para refinar 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 todos 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.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); y Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992).

Anticuerpos HER2 humanizados incluyen huMAb4D5-l, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 y huMAb4D5-8 o Trastuzumab (HERCEPTIN®) como se describe en la Tabla 3 de la Patente de los Estados Unidos 5,821,337 expresamente incorporada en esta solicitud por referencia; los anticuerpos 520C9 humanizados (WO93/21319) y 2C4 humanizado, como se describe en esta solicitud.

Para los propósitos de esta solicitud, "Trastuzumab," "HERCEPTIN®," y "huMAb4D5-8" se refieren a un anticuerpo que comprende secuencias de aminoácidos de cadena ligera y pesada en SEQ ID NOS. 13 y 14, respectivamente.

En esta solicitud, "Pertuzumab," "rhuMAb2C4," y "OMNITARG™" se refieren a un anticuerpo que comprende las secuencias de aminoácidos ligeras variables y pesadas variables en SEQ ID Nos. 3 y 4, respectivamente. Donde Pertuzumab es un anticuerpo de longitud total, éste preferiblemente comprende las secuencias de aminoácidos de cadena ligera y cadena pesada en SEQ ID NOS. 15 y 16, respectivamente.

Un "anticuerpo desnudo" es un anticuerpo (como se define en esta solicitud), que no se conjuga a una molécula heteróloga, tal como una fracción citotóxica o radiomarca.

Un anticuerpo "madurado por afinidad" es uno con una o más alteraciones en una o más regiones hipervariables del mismo, que resultan en una mejora en la afinidad del anticuerpo para el antígeno, comparada con un anticuerpo progenitor que no posee esa(s) alteración (alteraciones). Anticuerpos preferidos madurados por afinidad tendrán afinidades nanomolares o, incluso, picomolares para el antígeno diana. Anticuerpos madurados por afinidad se producen por procedimientos conocidos en el arte. Marks *et al.*, *Biotechnology*, 10:779-783 (1992) describe la maduración por afinidad por entremezcla de dominio VH y VL. Mutagénesis aleatoria de CDR y/o residuos de estructura alterna se describen en: Barbas, *et al.*, *Proc. Nat. Acad. Sci, USA* 91:3809-3813 (1994); Shier *et al.*, *Gene* 169:147-155 (1995); Yelton *et al.*, *J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); y Hawkins *et al.*, *J. Mol Biol* 226:889-896 (1992).

Un "anticuerpo agonista" es un anticuerpo que une y activa un receptor. Generalmente, la capacidad de activación de receptor del anticuerpo agonista será, como mínimo, cualitativamente similar (y puede ser esencialmente cuantitativamente similar) a la de un ligando agonista nativo del receptor. Un ejemplo de un anticuerpo agonista es uno que se une a un receptor en la superfamilia de receptores TNF, tal como DR5, e induce apoptosis de células que expresan el receptor TNF (por ejemplo, DR5). Análisis para determinar la inducción de apoptosis se describen en WO98/51793 y WO99/37684, las cuales se incorporan expresamente en esta solicitud por referencia.

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. En

incorporaciones preferidas, el anticuerpo se purificará (1) a más del 95% en peso del anticuerpo, según se determine por el método de Lowry y, muy preferiblemente 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 no estará presente por lo menos un componente del entorno natural del anticuerpo. De ordinario, sin embargo, el antagonista aislado se preparará en por lo menos un paso de purificación.

Un anticuerpo HER2 que "inhibe la dimerización HER más efectivamente que Trastuzumab" es uno que reduce o elimina los dímeros HER más efectivamente (por ejemplo por lo menos alrededor de 2 veces más efectivamente) que Trastuzumab. Preferiblemente, tal anticuerpo inhibe la dimerización HER2 por lo menos casi tan efectivamente como un anticuerpo seleccionado del grupo conformado por el anticuerpo monoclonal murino 2C4, un fragmento Fab del anticuerpo monoclonal murino 2C4, Pertuzumab, y un fragmento Fab de Pertuzumab. Se puede evaluar la inhibición de la dimerización HER estudiando los dímeros HER directamente, o evaluando la activación HER, o la señalización corriente abajo, que resulta de la dimerización HER, y/o evaluando el sitio de unión anticuerpo-HER2, etc. Análisis para cribar los anticuerpos con la capacidad de inhibir la dimerización HER más efectivamente que Trastuzumab se describen en Agus *et al., Cancer Cell* 2: 127-137 (2002) y WO01/00245 (Adams *et al.*). A modo de ejemplo únicamente, se puede analizar con respecto a la inhibición de la dimerización HER evaluando, por ejemplo, la inhibición de la formación de dímero HER (véase, por ejemplo, Fig. IA-B de Agus *et al., Cancer Cell* 2: 127-137 (2002); y WO01/00245); la reducción en la activación del ligando HER de células que expresan dímeros HER (WO01/00245 y Fig. 2A-B de Agus *et al., Cancer Cell* 2: 127-137 (2002), por ejemplo); bloqueo de unión de ligando HER a células

que expresan dímeros HER (WO01/00245, y Fig. 2E de Agus *et al., Cancer Cell* 2: 127-137 (2002), por ejemplo); inhibición del crecimiento celular de células cancerosas (por ejemplo, células MCF7, MDA-MD-134, ZR-75-1, MD-MB-175, T-47D), que expresan dímeros HER en la presencia (o ausencia) del ligando HER (WO01/00245 y Figs. 3A-D de Agus *et al., Cancer Cell* 2: 127-137 (2002), por ejemplo); inhibición de la señalización corriente abajo (por ejemplo, inhibición de la fosforilación de AKT dependiente de HRG, o inhibición de la fosforilación MAPK dependiente de HRG o TGFα) (véase, WO01/00245, y Fig. 2C-D de Agus *et al., Cancer Cell* 2: 127-137 (2002), por ejemplo). Se puede evaluar asimismo si el anticuerpo inhibe la dimerización HER estudiando el sitio de unión anticuerpo-HER2, por ejemplo, evaluando una estructura o modelo, tal como una estructura cristalina, del anticuerpo unido a HER2 (véase, por ejemplo, Franklin *et al., Cancer Cell* 5:317-328 (2004)).

El anticuerpo HER2 puede "inhibir la fosforilación de AKT dependiente de HRG" y/o inhibir "la fosforilación de MAPK dependiente de HRG o TGFα" más efectivamente (por ejemplo por lo menos 2 veces más efectivamente) que Trastuzumab (véase, Agus *et al., Cancer Cell* 2: 127-137 (2002) y WO01/00245, a modo de ejemplo).

El anticuerpo HER2 puede ser uno que "no inhibe el corte proteolítico del ectodominio HER2" (Molina *et al., Cancer Res.* 61:4744-4749(2001).

Un anticuerpo HER2 que "se une a un sitio heterodimérico de unión" de HER2, se une a residuos en el dominio II (y, de manera opcional, se une asimismo a residuos en otro de los dominios del dominio extracelular HER2, tal como los dominios I y III), y puede impedir estéricamente, por lo menos en alguna medida, la formación de un heterodímero HER2-EGFR, EGFR2-HER3, o HER2-HER4. Franklin *et al., Cancer Cell* 5:317-328 (2004) caracterizan la estructura cristalina de HER2-Pertuzumab, depositada ante el RCSB Protein Data Bank (ID Code IS78), que ilustra un anticuerpo ejemplar que se une al sitio heterodimérico de unión de HER2.

Un anticuerpo que "se une al dominio II" de HER2 se une a residuos en el dominio II y, opcionalmente, residuos en otro dominio(s) de HER2, tales como los dominios I y III. Preferiblemente el anticuerpo que se une al dominio II se une al empalme entre los dominios I, II y III de HER2.

Un "agente inhibidor de crecimiento", cuando se emplea en esta solicitud, se refiere a un compuesto o composición que inhibe el crecimiento de una célula, especialmente una célula cancerosa que expresa HER *in vitro* o *in vivo*. Por consiguiente, el agente inhibidor de crecimiento puede ser uno que reduce significativamente el porcentaje de células que expresan HER 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 de G1 y la detención de fase M. Bloqueadores clásicos de fase M incluyen las vincas (vincristina y vinblastina), taxanos e inhibidores topo II tales como doxorrubicina, epirrubicina, daunorrubicina, etopósido, y bleomicina. Aquellos agentes que detienen G1 pasan también a la detención de la fase S, por ejemplo, agentes alquilantes de ADN tales como tamoxifeno, prednisona, dacarbazina, mecloretamina, cisplatino, metotrexato, 5-fluorouracilo, y ara-C. Más información puede encontrarse en *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), especialmente página 13).

Ejemplos de anticuerpos "inhibidores de crecimiento" son aquellos que se unen a HER2 e inhiben el crecimiento de células cancerosas, que sobreexpresan HER2. Anticuerpos HER2 inhibidores de crecimiento preferidos inhiben el crecimiento de células de tumor de mama SK-BR-3 en un cultivo de células en más de 20% y, preferiblemente, más de 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, en donde la inhibición del crecimiento se determina seis días después de la exposición de las células SK-BR-3 al anticuerpo (véase Patente de los Estados Unidos No. 5,677,171 expedida el 14 de octubre de 1997). El análisis de inhibición

del crecimiento de las células SK-BR-3 se describe con más detalle en esa patente y más adelante en esta solicitud. El anticuerpo inhibidor de crecimiento preferido es una variante humanizada del anticuerpo monoclonal murino 4D5, por ejemplo, Trastuzumab.

Un anticuerpo que "induce apoptosis" es uno que induce la muerte celular programada, determinada por la unión de anexina V, fragmentación de ADN, contracción celular (***"cell shrinkage"***), dilatación del retículo endoplasmático, fragmentación celular, y/o formación de vesículas de membrana (denominadas cuerpos apoptóticos). Usualmente la célula es una que expresa el antígeno al cual se une el anticuerpo. Preferiblemente, la célula es una célula tumoral. Por ejemplo, puede medirse la translocación de la fosfatidil serina (PS) por unión de anexina; la fragmentación de ADN puede evaluarse mediante la formación escalonada de ADN; y la condensación nuclear/de cromatina junto con la fragmentación de ADN puede evaluarse por cualquier aumento en células hipodiploides. Preferiblemente, el anticuerpo que induce apoptosis es uno que resulta en alrededor de 2 a 50 veces, preferiblemente alrededor de 5 a 50 veces y, muy preferiblemente, alrededor de 10 a 50 veces, la inducción de unión de anexina, relativa a una célula no tratada, en un análisis de unión a anexina usando células que expresan un antígeno, al cual se une el anticuerpo. Ejemplos de anticuerpos que inducen apoptosis son los anticuerpos HER2 7C2 y 7F3, y ciertos anticuerpos DR5.

El "epítopo 2C4" es la región en el dominio extracelular de HER2 al cual se une el anticuerpo 2C4. Con el fin de cribar con respecto a anticuerpos que se unen al epítopo 2C4, puede llevarse a cabo un análisis de bloqueo cruzado rutinario, tal como el descrito en Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988). Alternativamente, puede realizarse mapeo de epítopos para evaluar si el anticuerpo se une al epítopo 2C4 de HER2. El epítopo 2C4 comprende residuos del dominio II en el dominio extracelular de HER2. 2C4 y Pertuzumab se unen al dominio extracelular de HER2 en el empalme de los dominios I, II y III. Franklin *et al., Cancer Cell* 5:317-328 (2004).

El "epítopo 4D5" es la región en el dominio extracelular de HER2 a la cual se unen el anticuerpo 4D5 (ATCC CRL 10463) y Trastuzumab. Este epítopo es cercano al dominio transmembrana de HER2, y está en el Dominio IV de HER2. Con el fin de cribar con respecto a anticuerpos que se unen al epítopo 4D5, puede llevarse a cabo un análisis de bloqueo cruzado rutinario, tal como el descrito en Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988). Alternativamente, puede realizarse mapeo de epítopos para evaluar si el anticuerpo se une al epítopo 4D5 de HER2 (por ejemplo, uno cualquiera o más residuos en la región desde alrededor del residuo 529 a alrededor del residuo 625, inclusive, de HER2).

El "epítopo 7C2/7F3" es la región en el término amino, en el Dominio I, del dominio extracelular de HER2, al cual se unen los anticuerpos 7C2 y/o 7F3 (cada uno depositado con la ATCC, véase más adelante). Con el fin de cribar con respecto a anticuerpos que se unen al epítopo 7C2/7F3, puede llevarse a cabo un análisis de bloqueo cruzado rutinario, tal como el descrito en Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988). Alternativamente, puede realizarse mapeo de epítopos para evaluar si el anticuerpo se une al epítopo 7C2/7F3 en HER2 (por ejemplo, uno cualquiera o más residuos en la región desde alrededor del residuo 22 a alrededor del residuo 53 de HER2).

"Tratamiento" se refiere tanto a tratamiento terapéutico como a medidas profilácticas o preventativas. Las personas en necesidad de tratamiento incluyen aquellas que ya sufren la enfermedad, así como aquellas en las cuales tiene que prevenirse la enfermedad. Por consiguiente, el paciente a tratar en esta solicitud puede haberse diagnosticado como portador de la enfermedad o puede estar predispuesto o ser susceptible a la enfermedad.

Los términos "cáncer" y "canceroso" o "maligno" se refieren a, o describen, la condición fisiológica en mamíferos, que se caracteriza típicamente por un crecimiento celular descontrolado. Ejemplos de cáncer

incluyen, pero no se limitan a, carcinoma incluidos adenocarcinoma, linfoma, blastoma (incluidos meduloblastoma y retinoblastoma), sarcoma (incluidos liposarcoma y sarcoma de células sinoviales), tumores neuroendocrinos (incluidos tumores carcinoides, gastrinoma, y insulinoma), mesotelioma, schwannoma (incluido neuroma acústico), meningioma, adenocarcinoma, melanoma, y leucemia o neoplasias malignas linfoides. Ejemplos más particulares de tales neoplasias malignas incluyen carcinoma epidermoide (por ejemplo, carcinoma epitelial), cáncer pulmonar incluidos cáncer de pulmón microcítico, cáncer de pulmón amicrocítico, adenocarcinoma del pulmón y carcinoma escamoso del pulmón, cáncer del peritoneo, cáncer hepatocelular, cáncer gástrico o del estómago, incluidos cáncer gastrointestinal, cáncer pancreático, glioblastoma, cáncer cervical, cáncer de ovarios, cáncer del hígado, cáncer de la vejiga, hematoma, cáncer de mama, cáncer de colon, cáncer rectal, cáncer colorrectal, carcinoma endometrial o uterino, carcinoma de las glándulas salivales, cáncer de riñón o renal, cáncer de próstata, cáncer de vulva, cáncer de la tiroides, carcinoma hepático, carcinoma anal, carcinoma del pene, cáncer testicular, cáncer esofágico, tumores del tracto biliar, así como cáncer de cabeza y cuello.

El término "cantidad efectiva" se refiere a una cantidad de un medicamento, efectiva para una enfermedad en el paciente. Donde la enfermedad es cáncer, la cantidad efectiva del medicamento puede reducir el número de células cancerosas; reducir el tamaño del tumor; inhibir (*es decir*, retardar en alguna medida y, preferiblemente, detener) la infiltración de células cancerosas en órganos periféricos; inhibir (*es decir*, retrasar en alguna medida y, preferiblemente, detener) la metástasis del tumor; inhibir en alguna medida el crecimiento del tumor; y/o 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. La cantidad efectiva puede ampliar la supervivencia libre de progresión, resultar en una respuesta objetiva (incluidas una respuesta parcial, PR, o respuesta

completa, CR), incrementar el tiempo total de supervivencia, y/o mejorar uno o más síntomas de cáncer.

Un "cáncer que sobreexpresa HER2" es uno que comprende células que tienen la proteína HER2 presente en su superficie celular.

Un cáncer que "sobreexpresa" un receptor HER es uno que tiene niveles significativamente más altos de un receptor HER, tal como HER2, 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 HER puede determinarse en un análisis diagnóstico o prognóstico evaluando niveles crecientes de la proteína HER presente en la superficie de una célula (*por ejemplo*, mediante un análisis inmunohistoquímico; IHC). Alternativa, o adicionalmente, se pueden medir niveles de ácido nucleico codificante de HER en la célula, *por ejemplo*, mediante hibridación *in situ* fluorescente (FISH; véase WO 98/45479 publicada en octubre de 1998), técnicas ***southern blotting***, o reacción en cadena de polimerasa (PCR), tal como PCR cuantitativa en tiempo real (RT-PCR). Se puede estudiar también la sobreexpresión del receptor HER midiendo el antígeno circulante (*por ejemplo*, dominio extracelular HER) en un fluido biológico tal como suero (véase, *por ejemplo*, Patente de los Estados Unidos 4,933,294 expedida el 12 de junio de 1990; WO 91/05264 publicada el 18 de abril de 1992; Patente de los Estados Unidos 5,401,638 expedida el 28 de marzo de 1995; y Sias *et al.*, *J. Immunol. Methods* 132: 73-80 (1990)). 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.

218

Recíprocamente, un cáncer que "no sobreexpresa el receptor HER2 receptor" es uno que no expresa niveles más altos que los normales de receptor HER, comparados con una célula no cancerosa del mismo tipo de tejido.

Un cáncer que "sobreexpresa" un ligando HER es uno que produce niveles significativamente más altos de ese ligando, comparados 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 del ligando HER puede determinarse diagnósticamente evaluando niveles del ligando (o ácido nucleico que lo codifica) en un paciente, por ejemplo, en una biopsia del tumor o mediante varios análisis diagnósticos tales como IHC, FISH, ***southern blotting***, PCR o análisis *in vivo* descritos anteriormente.

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*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} 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, incluidos sus fragmentos y/o variantes.

Un "agente quimioterapéutico" es un compuesto químico útil en el tratamiento de enfermedades como cáncer. Ejemplos de agentes quimioterapéuticos incluyen agentes alquilantes tales como tiotepa y ciclosfosfamida (CYTOXAN™); 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); delta-9-tetrahidrocannabinol (dronabinol, MARINOL®); beta-lapacona; lapacol; colquicinas; ácido betulínico; una camptotecina (incluido el análogo sintético topotecán (HYCAMTIN), CPT-11

(irinotecán, CAMPTOSAR®), acetilcamptotecina, escopolectina, y 9-aminocamptotecina); briostatina; calistatina; CC-1065 (incluidos sus análogos sintéticos adozelesina, carzelesina y bizelesina); podofilotoxina; ácido podofilínico; tenipósido; 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 ranimustina; antibióticos tales como los antibióticos de enediíno (*por ejemplo*, caliqueamicina, especialmente caliqueamicina γ_1^I y caliqueamicina Ω^I_1 (véase, *por ejemplo*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dinemicina, incluida dinemicina A; una esperamicina; así como cromóforo de neocarzinostatina y cromóforos antibióticos relacionados de enediíno cromoproteína), aclacinomisinas, actinomicina, autramicina, azaserina, bleomicinas, cactinomicina, carabicina, carminomicina, carzinofilina, cromomicinas, dactinomicina, daunorubicina, detorubicina, 6-diazo-5-oxo-L-norleucina, doxorubicina (incluidas ADRIAMYCIN®, morfolino-doxorubicina, cianomorfolino-doxorrubicina, 2-pirrolino-doxorrubicina y deoxidoxorrubicina, inyección de liposomas de HCl de clodoxorrubicina (DOXIL®), doxorrubicina liposómica TLC D-99 (MYOCET®), doxorrubicina liposómica peglilada (CAELYX®), y deoxidoxorrubicina), epirrubicina, esorrubicina, idarrubicina, marcelomicina, mitomicinas tales como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorrubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatina, zorrubicina; anti-metabolitos tales como metotrexato, gemcitabina (GEMZAR®), tegafur (UFTORAL®), capecitabina (XELODA®), una epotilona, 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; anti-adrenales tales como aminoglutetimida, mitotano, trilostano; reabastecedor de ácido fólico tal como ácido frolínico; aceglatona; glucósido de aldofosfamida; ácido aminolevulínico; eniluracil; amsacrina; bestrabucil; bisantreno; edatraxato; defofamina; demecolcina; diazicuona; elfornithine; acetato de eliptinio; una epotilona; etoglucid; nitrato de galio; hidroxiurea; lentinan; lonidainina; maitansinoides tales como maitansina y ansamitocinas; mitoguazona; mitoxantrona; mopidamol; nitracrina; pentostatina; phenamet; pirarubicina; losoxantrona; 2-etilhidrazida; procarbazina; PSKL® complejo de polisacáridos (JHS Natural Products, Eugene, OR); razoxano; rizoxina; sizofirano; espirogermanio; ácido tenuazónico; triazicuona; 2,2',2"-triclorotrietilamina; tricotecenos (especialmente T-2 toxina, verracurina A, roridina A y anguidina); uretano; dacarbazina; mannomustina; mitobronitol; mitolactol; pipobroman; gacitosina; arabinósido ("Ara-C"); tiotepa; taxoide, *por ejemplo*, paclitaxel (TAXOL®, formulación de nanopartículas diseñadas con albúmina de paclitaxel (AMBRAXANE™) y doxetaxel (TAXOTERE®); clorambucil; 6-tioguanina; mercaptopurina; metotrexato; agentes de platino tales como cisplatino, oxaliplatino y carboplatino; vincas, que impiden la polimerización de tubulina de formar microtúbulos, incluida vinblastina (VELBAN®), vincristina (ONCOVIN®), vindesina (ELDISINE®, FILDESIN®), y vinorelbina (NAVELBINE®); etopósido (VP-16); ifosfamida; mitoxantrona; leucovovina; novantrona; edatrexato; daunomicina; aminopterina; ibandronato; inhibidor de toposiomerasa RFS 2000; difluorometilornitina (DMFO); retinoides tales como ácido retinoico, incluido bexaroteno (TARGRETIN®); bisfosfonatos tales como clodronato (por ejemplo, BONEFOS® u OSTAC®), etidronato (DIDROCAL®), NE-58095, ácido zoledrónico/zoledronato (ZOMETA®), alendronato (FOSAMAX®), pamidronato (AREDIA®), tiludronato (SKELID®), o risedronato (ACTONEL®); troxacitabina (un análogo de 1,3-dioxolano

nucleósido citosina); oligonucleótidos complementarios, particularmente aquellos que inhiben la expresión de genes en las rutas de señalización implicadas en la proliferación celular aberrante, tales como, por ejemplo, PKC-alpha, Raf, H-Ras, y receptor del factor de crecimiento epidérmico (EGF-R); vacunas tales como la vacuna THERATOPE® y vacunas de terapia génica, por ejemplo, la vacuna ALLOVECTIN®, la vacuna LEUVECTIN®, y la vacuna VAXID®; inhibidor de topoisomerasa 1 (por ejemplo, LURTOTECAN®); rmRH (por ejemplo, ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (Pfizer); perifosina, inhibidor de COX-2 (por ejemplo, celecoxib o etoricoxib), inhibidor de proteosomas (por ejemplo, PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R1 1577); orafenib, ABT510; inhibidor de Bcl-2 tal como oblimersen sódico (GENASENSE®); pixantrona; inhibidores EGFR (véase definición más adelante); inhibidores de tirosina cinasa (véase definición más adelante); y sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores; así como combinaciones de dos o más de los anteriores tales como CHOP, una abreviatura para una terapia combinada de ciclofosfamida, doxorrubicina, vincristina, y prednisolona, y FOLFOX, una abreviatura para un régimen de tratamiento con oxaliplatino (ELOXATIN™) combinado con 5-FU y leucovovina.

También incluidos en esta definición se encuentran agentes anti-hormonales que actúan para regular o inhibir la acción de las hormonas en tumores, tales como anti-estrógenos con perfil mixto agonista/antagonista, incluidos, tamoxifeno (NOLVADEX®), 4-hidroxitamoxifeno, toremifeno (FARESTON®), idoxifeno, droloxifeno, raloxifeno (EVTSTA), trioxifeno, keoxifeno, y moduladores selectivos de receptor de estrógeno (SERM) tales como SERM3; anti-estrógenos puros sin propiedades agonistas, tales como fulvestrant (FASLODEX®), y EM800 (tales agentes pueden bloquear la dimerización del receptor de estrógeno (ER), inhibir la unión de ADN, aumentar la rotación de ER, y/o suprimir los niveles de ER); inhibidores de aromatasa, incluidos los inhibidores de aromatasa esteroides tales como formestano y exemestano (AROMASIN®), e

inhibidores de aromatasa no esteroides tales como anastrazol (ARIMIDEX®), letrozol (FEMARA®) y aminoglutetimida, y otros inhibidores de aromatasa incluidos vorozol (RIVISOR®), acetato de megestrol (MEGASE®), fadrozol, imidazol; agonistas de luliberina, incluidas leuprolida (LUPRON® y ELIGARD®), goserelina, buserelina, y tripterelina; esteroides sexuales, incluidas progestinas tales como acetato de megestrol y acetato de medroxiprogesterona, estrógenos tales como dietilstilbestrol y premarina, y andrógenos/retinoides tales como fluoximesterona, ácido transretionico y fenretinida; onapristona; anti-progesteronas; reguladores por disminución de receptor de estrógeno (ERD); anti-andrógenos tales como flutamida, nilutamida y bicalutamida; testolactona; y sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores; así como combinaciones de dos o más de los anteriores.

Tal como se emplea en esta solicitud, el término "fármaco dirigido a EGFR" se refiere a un agente terapéutico que se une a EGFR y, opcionalmente, inhibe la activación de EGFR. Ejemplos de tales agentes incluyen anticuerpos y pequeñas moléculas que se unen a EGFR. Ejemplos de anticuerpos que se unen a EGFR incluyen MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (véase, Patente de los Estados Unidos No. 4,943, 533, Mendelsohn *et al.*) y sus variantes, tales como 225 quimerizado (C225 o Cetuximab; ERBUTIX®) y 225 humano reconfigurado (H225) (véase, WO 96/40210, Imclone Systems Inc.); anticuerpos que se unen EGFR mutante tipo II (Patente de los Estados Unidos No. 5,212,290); anticuerpos humanizados y quiméricos que unen EGFR, como se describe en Patente de los Estados Unidos No. 5,891,996; y anticuerpos humanos que unen EGFR, tales como ABX-EGF (véase WO98/50433, Abgenix). El anticuerpo anti-EGFR puede conjugarse con un agente citotóxico, generando en esa forma un inmunoconjugado (véase, por ejemplo, EP659,439A2, Merck Patent GmbH). Ejemplos de moléculas pequeñas que se unen a EGFR incluyen ZD 1839 o Gefitinib (IRESSA™; Astra Zeneca),

CP-358774 o Erlotinib HCL (TARCEVA™; Genentech/OSI) y AG1478, AG1571 (SU 5271; Sugen).

Un "inhibidor de tirosina cinasa" es una molécula que inhibe en cierta medida la actividad de una tirosina cinasa tal como un receptor HER. Ejemplos de tales inhibidores incluyen los fármacos dirigidos a EGFR anotados en el párrafo precedente, así como el inhibidor de tirosina cinasa HER2 de molécula pequeña tal como TAK165 disponible de Takeda, inhibidores dual-HER tales como EKB-569 (disponible de Wyeth), el cual se une preferencialmente a EGFR pero inhibe las células que sobreexpresan a los dos HER2 & EGFR, GW572016 (disponible de Glaxo) un inhibidor de tirosina cinasa HER2 y EGFR oral, y PKI- 166 (disponible de Novartis); inhibidores pan-HER tales como canertinib (CI-1033; Pharmacia); inhibidores de Raf-1 tales como el agente complementario ISIS-5132, disponible de ISIS Pharmaceuticals, que inhibe la señalización Raf-1; inhibidores de TK no dirigida a HER tales como mesilato de Imatinib (Gleevac™) disponible de Glaxo; inhibidor de cinasa I regulada por MAPK extracelular CI-1040 (disponible de Pharmacia); quinazolinas, tales como PD 153035, 4-(3-cloroanilino)-quinazolina; piridopirimidinas; pirimidopirimidinas; pirrolopirimidinas, tales como CGP 59326, CGP 60261 y CGP 62706; pirazolopirimidinas, 4-(fenilamina)-7H-pirrolo[2,3-d]pirimidinas; curcumina (diferuloil metano, 4,5-bis (4-fluoroanilino)ftalimida); tirfostinas que contienen fracciones nitrotiofeno; PD-0183805 (Warner-Lamber); moléculas complementarias (por ejemplo, aquellas que se unen a ácido nucleico que codifica HER); quinoxalinas (Patente de los Estados Unidos No. 5,804,396); trifostinas (Patente de los Estados Unidos No. 5,804,396); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); inhibidores de pan-HER tales como CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); mesilato de Imatinib (Gleevac; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-IC11 (Imclone); o como se describe en cualquiera de las siguientes publicaciones de patente: Patente de los

Estados Unidos No. 5,804,396; WO99/09016 (American Cyanimid); WO98/43960 (American Cyanamid); WO97/38983 (Warner Lambert); WO99/06378 (Warner Lambert); WO99/06396 (Warner Lambert); WO96/30347 (Pfizer, Inc); WO96/33978 (Zeneca); WO96/3397 (Zeneca); y WO96/33980 (Zeneca).

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

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; 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 (CSF) tales como CSF de macrófago (M-CSF); CSF de granulocito-macrófago (GM-CSF); y CSF de granulocito (G-CSF); interleucinas (IL) tales como IL-1, IL-1α, IL-2, IL-

225

3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; un factor de necrosis tumoral tal como TNF-α o 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 natural.

Preferiblemente, el anticuerpo que se formula es esencialmente puro y, en forma deseable, esencialmente homogéneo (es decir, libre de proteínas contaminantes, etc). Anticuerpo "esencialmente puro" significa una composición que comprende por lo menos alrededor de 90% en peso del anticuerpo, basándose en el peso total de la composición, preferiblemente por lo menos alrededor de 95% en peso. Anticuerpo "esencialmente homogéneo" significa una composición que comprende por lo menos alrededor de 99% en peso de anticuerpo, basándose en el peso total de la composición.

Un "marcador de superficie de linfocito B" o "antígeno de superficie de linfocito B" en esta solicitud es un antígeno expresado en la superficie de un linfocito B que puede tomar como diana con un anticuerpo que se une a él. Marcadores ejemplares de superficie de linfocito B incluyen los marcadores de superficie de leucocito CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 y CD86 (para descripciones, véase The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.*, Academic Press, Harcourt Brace & Co., New York). Otros marcadores de superficie de linfocito B incluyen RP105, FcRH2, CR2 de linfocito B, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, y 239287. El marcador de superficie de linfocito B de interés particular en esta solicitud se expresa preferencialmente en linfocitos B, comparado con otros tejidos distintos de linfocitos B de un mamífero, y pueden expresarse en linfocitos B

precursores y linfocitos B maduros. El marcador de superficie de linfocito B preferido en esta solicitud es CD20 ó BR3.

El antígeno "CD20", o "CD20," es una fosfoproteína no glucosilada de alrededor de 35-kDa, hallada en la superficie de más del 90% de los linfocitos B de la sangre periférica u órganos linfoides. El CD20 está presente tanto en los linfocitos B normales como también en los linfocitos B malignos, pero no se expresa en células madre. Otros nombres para CD20 en la literatura incluyen "antígeno restringido por linfocito B" y "Bp35". El antígeno CD20 se describe en Clark *et al., Proc. Natl Acad. Sci. (USA)* 82:1766 (1985), por ejemplo.

Puramente para los propósitos de esta solicitud, "2H7 humanizado" se refiere a una variante humanizada del anticuerpo 2H7 cuyas secuencias CDR se divulgan en la Patente de los Estados Unidos No. 5,500,362 (Figs. 5 y 6), expresamente incorporada en esta solicitud por referencia. Ejemplos de anticuerpos 2H7 humanizados en esta solicitud incluyen las variantes descritas en WO2004/056312, también expresamente incorporada en esta solicitud por referencia, así como otras variantes, incluidas, pero no limitadas a: 2H7v16, 2H7v31, 2H7v73, 2H7v75, 2H7v96, 2H7v114, 2H7v115, 2H7v116, 2H7v138, 2H7v477, 2H7v375, etc.

En una incorporación, el anticuerpo 2H7 humanizado comprende una, dos, tres, cuatro, cinco o seis de las siguientes secuencias CDR:
secuencia CDR L1 RASSSVSYXH en donde X es M o L (SEQ ID No. 67), por ejemplo SEQ ID No.57 (Fig. 18A),
secuencia CDR L2 de SEQ ID No. 58 (Fig. 18A),
secuencia CDR L3 QQWXFNPPT en donde X es S o A (SEQ ID No. 68), por ejemplo SEQ ID No. 59 (Fig. 18A),
secuencia CDR H1 de SEQ ID No. 60 (Fig. 18B),
secuencia CDR H2 de AIYPGNGXTSYNQKFKG en donde X es D o A (SEQ ID No. 69), por ejemplo SEQ ID No. 61 (Fig. 18B), y
secuencia CDR H3 de VVYYSXXYWYFDV en donde la X en la posición 6 es N, A, Y, W o D, y la X en la posición 7 es S o R (SEQ ID No. 70), por ejemplo SEQ ID No. 62 (Fig. 18B).

Las secuencias CDR anteriores están generalmente presentes en las secuencias de estructura alterna pesadas variables y ligeras variables humanas, tales como sustancialmente los residuos FR de consenso humanos del subgrupo I kappa de cadena ligera humano ($V_{LK}I$), y sustancialmente los residuos FR de consenso humanos del subgrupo III de cadena pesada humano (V_HIII). Véase también WO 2004/056312 (Lowman *et al.*).

La región pesada variable puede empalmarse a una región constante de cadena IgG humana, en donde la región puede ser, por ejemplo, IgG1 o IgG3, incluidas regiones constantes de variantes y secuencias naturales.

En una incorporación preferida, tal anticuerpo comprende la secuencia de dominio pesado variable de SEQ ID No. 29 (v16, como se muestra en Fig. 18B), comprendiendo también opcionalmente la secuencia de dominio ligero variable de SEQ ID No. 26 (v16, como se muestra en Fig. 18A), la cual comprende opcionalmente una o más sustituciones de aminoácidos en las posiciones 56, 100, y/o 100a, por ejemplo D56A, N100A o N100Y, y/o S100aR en el dominio pesado variable, y una o más sustituciones de aminoácidos en las posiciones 32 y/o 92, por ejemplo M32L y/o S92A, en el dominio ligero variable. Preferiblemente, el anticuerpo es un anticuerpo intacto que comprende las secuencias de aminoácidos de cadena ligera de SEQ ID Nos. 63 ó 64, y las secuencias de aminoácidos de cadena pesada de SEQ ID No. 65, 66, 71 ó 72.

Un anticuerpo 2H7 humanizado preferido es ocrelizumab (Genentech).

El anticuerpo en esta solicitud puede comprender además por lo menos una sustitución de aminoácido en la región Fc que mejore la actividad ADCC, tal como una en donde las sustituciones de aminoácido están en las posiciones 298, 333, y 334, preferiblemente S298A, E333A, y K334A, usando la numeración Eu de residuos de cadena pesada. Véase asimismo Patente de los Estados Unidos No. 6,737,056B1, Presta.

Cualquiera de estos anticuerpos puede comprender por lo menos una sustitución en la región Fc, que mejore la unión FcRn o semivida sérica, por ejemplo una sustitución en la posición de cadena pesada 434, tal como

N434W. Véase también Patente de los Estados Unidos No. 6,737,056B1, Presta.

Cualquiera de estos anticuerpos puede comprender además por lo menos una sustitución en la región Fc que aumente la actividad CDC, por ejemplo, que comprenda por lo menos una sustitución en la posición 326, preferiblemente K326A o K326W. Véase igualmente Patente de los Estados Unidos No. 6,528,624B 1 (Idusogie *et al.*).

Algunas variantes preferidas de 2H7 humanizado son aquellas que comprenden el dominio ligero variable de SEQ ID No. 26 y el dominio pesado variable de SEQ ID No. 29, incluidas aquellas con o sin substituciones en una región Fc (si está presente), y aquellas que comprenden un dominio pesado variable con alteración N100A; o D56A y N100A; o D56A, N100Y, y S100aR; en SEQ ID No. 29 y un dominio ligero variable con alteración M32L; o S92A; o M32L y S92A; en SEQ ID No. 26.

M34 en la cadena pesada variable de 2H7v16 se ha identificado como una fuente potencial de estabilidad de anticuerpo y es otro candidato potencial para sustitución.

En un resumen de varias incorporaciones preferidas de la invención, la región variable de variantes basadas en 2H7v16 comprende las secuencias de aminoácidos de v16, excepto en las posiciones de sustituciones de aminoácido que se indican en la siguiente Tabla. A menos que se indique lo contrario, las variantes 2H7 tendrán la misma cadena ligera que la de v16.

Variantes Ejemplares de Anticuerpo 2H7 Humanizado

Versión 2H7	Cambios de Cadena Pesada (V_H)	Cambios de Cadena Ligera (V_L)	Cambios Fc
16 para referencia			
31	-		S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	

114	D56A, N100A	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K334A, K322A
138	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
477	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
375	-	-	K334L
588	-	-	S298A, E333A, K334A, K326A
511	D56A, N100Y,	M32L, S92A	S298A, E333A, K334A, K326A

Un 2H7 humanizado preferido comprende la secuencia de dominio ligero variable 2H7v16:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID No. 26);

y la secuencia de dominio pesado variable 2H7v16:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID No. 29).

Donde el anticuerpo 2H7v16 humanizado es un anticuerpo intacto, éste puede comprender la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID No. 63);

y la secuencia de aminoácidos de cadena pesada de SEQ ID No. 65 o:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 71).

Otro anticuerpo 2H7 humanizado preferido comprende la secuencia de dominio ligero variable 2H7v511:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID No. 73)

y la secuencia de dominio pesado variable 2H7v511:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS (SEQ ID No. 74).

En donde el anticuerpo 2H7v511 humanizado es un anticuerpo intacto, éste puede comprender la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID No. 64)

y la secuencia de aminoácidos de cadena pesada de SEQ ID No. 66 o:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAA
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 72).

Una "neoplasia maligna de linfocitos B" en esta solicitud comprende linfoma no hodgkiniano (NHL), incluido NHL de bajo grado/folicular, NHL linfocítico pequeño (SL), NHL de grado intermedio/folicular, NHL difuso de grado intermedio, NHL inmunoblástico de grado elevado, NHL linfoblástico de grado elevado, NHL de microcitos no segmentados de grado elevado, linfoma de células del manto; linfoma relacionado con SIDA, y macroglobulinemia de Waldenstrom; leucemia, incluida leucemia linfoblástica aguda (ALL), leucemia linfocítica crónica (CLL), tricoleucemia y leucemia mieloblástica crónica, y otras neoplasias malignas hematológicas. Tales neoplasias malignas pueden tratarse con anticuerpos dirigidos contra marcadores de superficie de linfocitos B, tales como CD20.

El término "linfoma no hodgkiniano" o "NHL", como se usa en esta solicitud, se refiere a un cáncer del sistema linfático, distinto de los linfomas hodgkinianos. Por lo general, los linfomas hodgkinianos pueden distinguirse de los linfomas no hodgkinianos por la presencia de células de Reed-Sternberg en linfomas hodgkinianos y la ausencia de dichas células en linfomas no hodgkinianos. Ejemplos de linfomas no hodgkinianos comprendidos por el término, como se emplea en esta solicitud, incluyen cualquiera que sería identificado como tal por un experto en el arte (por ejemplo, un oncólogo o patólogo), de acuerdo con esquemas de clasificación conocidos en el arte, tal como el esquema Revised European-American Lymphoma (REAL) como se describe en Color Atlas of Clinical Hematology, Third Edition; A. Victor Hoffbrand and John E. Pettit (eds.)

(Harcourt Publishers Limited 2000) (véase, en particular Fig. 11.57, 11.58 y/o 11.59). Ejemplos más específicos incluyen, pero no se limitan a, NHL de recaída o resistente a tratamiento, NHL de bajo grado de primera línea, NHL de Fase III/IV, NHL resistente a la quimioterapia, leucemia linfoblástico de precursores B y/o linfoma, linfomas linfocíticos pequeños, leucemia linfocítica crónica de linfocitos B y/o leucemia prolinfocítica y/o linfoma linfocítico pequeño, linfoma prolinfocítico de linfocitos B, inmunocitoma y/o linfoma linfoplasmacítico, linfoma de linfocitos B de zona marginal, linfoma de zona marginal esplénica, linfona MALT – zona marginal extranodal, linfoma de zona marginal nodal, tricoleucemia, plasmacitoma y/o mieloma de células plasmáticas, linfoma de bajo grado/folicular, NHL de grado intermedio/folicular, linfoma de células de manto, linfoma del centro del folículo (folicular), NHL difuso de grado intermedio, linfoma de linfocitos B grandes difusos, NHL crónico activo (incluido NHL de primera línea crónico activo y NHL de recaída crónico activo), NHL recurrente o resistente a transplante de células madre autólogo, linfoma de linfocitos B grandes mediastinales primarios, linfoma de efusión primario, NHL inmunoblástico de grado alto, NHL linfoblástico de grado alto, NHL de células no segmentadas de alto grado, NHL de enfermedad de tumor sólido (***"bulky disease NHL"***), linfoma de Burkitt, leucemia linfoblástica de linfocitos T (periféricos) precursores y/o linfoma, linfoma de linfocitos T en adulto y/o leucemia, leucemia linfocítica crónica de linfocitos T y/o leucemia prolinfocítica, leucemia linfocítica granular grande, micosis fungoides y/o síndrome de Sezary, linfoma (tipo nasal) de linfocitos T/citolíticos naturales extranodales, linfoma de linfocitos T tipo enteropatía, linfoma de linfocitos T hepatoesplénico, linfoma de linfocitos T tipo paniculitis subcutánea, linfomas de la piel (cutáneos), linfoma de células grandes anaplásicas, linfoma angiocéntrico, linfoma de linfocitos T intestinales, linfoma de linfocitos T (no especificado en otra forma) periféricos y linfoma de linfocitos T angioinmunoblástico.

Una "enfermedad autoinmunitaria" contemplada aquí es una enfermedad o trastorno que se origina y dirige contra los propios tejidos de

un individuo, o un co-segregado o manifestación o afección resultante del mismo. Ejemplos de enfermedades o trastornos autoinmunitarios incluyen, pero no se limitan a artritis (artritis reumatoide, artritis reumatoide en jóvenes, osteoartritis, artritis psoriásica y espondilitis anquilosante), psoriasis, dermatitis, incluida dermatitis atópica; urticaria idiopática crónica, incluida urticaria autoinmunitaria crónica, polimiositis/dermatomiositis, necrólisis epidérmica tóxica, escleroderma (incluido escleroderma sistémico), esclerosis tal como esclerosis sistémica progresiva, síndrome de intestino irritable (IBD) (por ejemplo, enfermedad de Crohn, colitis ulcerosa, síndrome de intestino irritable autoinmunitario), *pyoderma gangrenosum*, *erythema nodosum*, colangitis esclerosante primaria, episcleritis), síndrome disneico, incluido síndrome disneico en adultos (ARDS), meningitis, enfermedades mediadas por IgE tales como anafilaxis y rinitis alérgica y atópica, encefalitis tal como encefalitis de Rasmussen, uveítis o uveítis autoinmunitaria, colitis tales como colitis microscópica y colitis colágena, glomerulonefritis (GN) tales como GN membranosa (neuropatía membranos), GN membranosa idiopática, GN proliferativa membranosa (MPGN), incluidas GN Tipo I y Tipo II, y de rápida evolución, afecciones alérgicas, reacción alérgica, eczema, asma, estados que involucran la infiltración de linfocitos T y respuestas inflamatorias crónicas, arterioesclerosis, miocarditis autoinmunitaria, deficiencia de adhesión de leucocitos, lupus eritematoso sistémico (SLE) tal como SLE cutáneo, lupus eritematoso cutáneo sub-agudo, lupus (incluidas nefritis, cerebritis, pediátrico, no renal, discoide, alopecia), diabetes sacarina de aparición en jóvenes (tipo I), incluida diabetes sacarina insulinodependiente pediátrica (IDDM), diabetes sacarina (tipo II) de aparición en adultos, esclerosis múltiple (MS) tal como MS espino-óptica, respuestas inmunitarias asociadas con hipersensibilidad aguda y diferida mediada por citocinas y linfocitos T, tuberculosis, sarcoidosis, granulomatosis incluida granulomatosis linfomatoide, granulomatosis de Wegener, agranulocitosis, vasculitis (incluidas vasculitis de vasos grandes (incluidas *polymyalgia rheumatica* y arteritis de células gigantes (de

Takayasu)), vasculitis de vasos medianos (incluidas la enfermedad de Kawasaki y *polyarteritis nodosa*), Vasculitis SNC, y vasculitis necrosante sistémica, vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (ANCA), tal como vasculitis o síndrome de Churg-Strauss (CSS)), arteritis temporal, anemia aplásica, anemia positiva de Coombs, anemia de Diamond Blackfan, anemia hemolítica o anemia hemolítica inmunitaria incluida anemia hemolítica autoinmunitaria (AIHA), anemia perniciosa, aplasia de glóbulos rojos puros (PRCA), deficiencia de factor VIII, hemofilia A, neutropenia autoinmunitaria, pancitopenia, leucopenia, enfermedades que involucran diapédesis de leucocitos, trastornos inflamatorios del SNC, síndrome de lesión de órganos múltiples, enfermedades mediadas por complejo antígeno-anticuerpo, enfermedad de membrana basal anti-glomerular, síndrome de anticuerpo anti-fosfolípido, neuritis alérgica, enfermedad de Bechet o Behçet, síndrome de Castleman, síndrome de Goodpasture, síndrome de Reynaud, síndrome de Sjögren, síndrome de Stevens-Johnson, penfigoide tal como penfigoide vesicular, pénfigo (incluidos *pemphigus vulgaris*, *foliaceus*, y penfigoide *pemphigus mucus-membrane*), poliendocrinopatías autoinmunitarias, enfermedad de Reiter, nefritis de complejo inmunitario, neuropatía crónica tal como polineuropatías IgM o neuropatía mediada por IgM, trombocitopenia (desarrollada por pacientes con infarto del miocardio, por ejemplo), incluidas púrpura trombocitopénica trombótica (TTP) y trombocitopenia autoinmunitaria o mediada inmunitariamente, tal como púrpura trombocitopénica idiomática (ITP), incluida ITP crónica o aguda, enfermedad autoinmunitaria de los testículos y ovario, incluidas orquitis autoinmunitarias y ooforitis, hipotiroidismo primario; enfermedades endocrinas autoinmunitarias incluida tiroiditis tal como tiroiditis autoinmunitaria, tiroiditis crónica (tiroiditis de Hashimoto), o tiroiditis subaguda, enfermedad tiroide autoinmunitaria, hipotiroidismo idiopático, enfermedad de Addison, enfermedad de Grave, síndromes poliglandulares tales como síndromes poliglandulares autoinmunitarios (o síndromes de endocrinopatía poliglandular), síndromes paraneoplásicos, incluidos

síndromes paraneoplásicos neuronales, tales como síndrome miasténico de Lambert-Eaton o síndrome de Eaton-Lambert, síndrome de hombre rígido o persona rígida, encefalomielitis tal como encefalomielitis alérgica, miastenia grave, degeneración cerebelar, encefalitis límbica o del tronco cerebral, neuromiotonía, síndrome de opsoclonus u opsoclonus myoclonus (OMS), y neuropatía sensorial, síndrome de Sheehan, hepatitis autoinmunitaria, hepatitis crónica, hepatitis lupoide, hepatitis activa crónica o hepatitis activa crónica autoinmunitaria, neumonitis intersticial linfoide, bronchiolitis obliterans (no transplante) vs NSDP, síndrome de Guillain-Barré, enfermedad de Berger (neuropatía IgA), cirrosis biliar primaria, esprúe celíaco (enteropatía del gluten), esprúe resistente a tratamiento, dermatitis herpetiformis, crioglobulinemia, esclerosis lateral amiotrófica (ALS; enfermedad de Lou Gehrig), arteriopatía coronaria, enfermedad autoinmunitaria del oído interno (AIED), o pérdida autoinmunitaria del oído, síndrome opsoclonus myoclonus (OMS), policondritis tal como policondritis resistente a tratamiento, proteinosis alveolar pulmonar, amiloidosis, hepatitis de células gigantes, escleritis, una linfocitosis no cancerosa, una lifocitosis primaria, que incluye linfocitosis de linfocitos B monoclonales (por ejemplo, gammopatía monoclonal benigna y gammopatía monoclonal de significación indeterminada, MGUS), neuropatía periférica, síndrome paraneoplásico, enfermedades que involucran una disfunción en el canal iónico tales como epilepsia, migraña, arritmia, trastornos musculares, sordera, ceguera, parálisis periódica, y enfermedades que involucran una disfunción en el canal iónico del SNC, autismo, miopatía inflamatoria, glomerulosclerosis segmental focal (FSGS), oftalmopatía endocrina, uveoretinitis, trastorno hematológico autoinmunitario, fibromialgia, falla endocrina múltiple, síndrome de Schmidt, adrenalitis, atrofia gástrica, demencia presenil, enfermedades desmielinizantes, síndrome de Dressler, alopecia areata, síndrome de CREST (calcinosis, fenómeno de Raynaud, dismotilidad esofágica, esclerodactilia, y telangiectasia), infertilidad masculina y femenina, espondilitis anquilosante, enfermedad del tejido conectivo mixto,

enfermedad de Chagas, fiebre reumática, aborto recurrente, neumonitis (*"farmer's lung"*), eritema multiforme, síndrome pos-cardiotomía, síndrome de Cushing, pulmón de criadores de aves (*"bird-fancier's lung"*), síndrome de Alport, alveolitis tal como alveolitis alérgica y alveolitis fibrótica, enfermedad pulmonar intersticial, reacción por transfusión, lepra, malaria, leishmaniasis, kipanosomiasis, esquistosomiasis, ascariasis, aspergilosis, síndrome de Sampter, síndrome de Caplan, dengue, endocarditis, fibrosis endomiocardial, endoftalmitis, erythema elevatum y diutinum, erythroblastosis fetalis, faciítis eosinófila, síndrome de Shulman, síndrome de Felty, flariasis, ciclitis tal como ciclitis crónica, ciclitis heterocrónica, o ciclitis de Fuch, púrpura de Henoch-Schönlein, infección por virus de inmunodeficiencia humana (VIH), infección de echovirus, cardiomiopatía, enfermedad de Alzheimer, infección por parvovirus, infección por el virus de la rubéola, síndromes pos-vacunación, infección por rubéola congénita, infección por el virus de Epstein-Barr, paperas, síndrome de Evan, falla gonadal autoinmunitaria, corea de Sydenham, nefritis pos-estreptocócica, *thromboangitis ubiterans*, tirotoxicosis, tabes dorsalis, y polimialgia de células gigantes.

La "superfamilia de receptores de factor de necrosis tumoral" o "superfamilia de receptores TNF" en esta solicitud se refiere a polipéptidos de receptor unidos por citocinas en la familia TNF. Generalmente, estos receptores son receptores transmembrana tipo I con una o más secuencias repetidoras ricas en cisteína en su dominio extracelular. La superfamilia de receptores TNF puede subdividirse aún más en (1) receptores de muerte; (2) receptores señuelo; y (3) receptores de señalización que carecen de dominios de muerte. Los "receptores de muerte" contienen en su región citoplásmica o intracelular un "dominio de muerte", es decir, una región o secuencia que actúa para transducir señales en la célula que pueden resultar en apoptosis o en inducción de ciertos genes. Los "receptores señuelo" carecen de un dominio de muerte funcional y son incapaces de transducir señales que resultan en apoptosis. Ejemplos de citocinas en la familia del gen TNF incluyen el Factor-α de Necrosis Tumoral (TNF-α), el Factor-β de

Necrosis Tumoral (TNF-β o linfotoxina), el ligando CD30, el ligando CD27, el ligando CD40, el ligando OX-40, el ligando 4-1BB, el ligando Apo-1 (también mencionado como ligando Fas o ligando CD95), el ligando Apo-2 (también mencionado como TRAIL), el ligando Apo-3 (también mencionado como TWEAK), osteoprotegerina (OPG), APRIL, el ligando RANK (también mencionado como TRANCE), y TALL-1 (también mencionado como BlyS, BAFF o THANK). Ejemplos de receptores en la superfamilia de receptores TNF incluyen: Receptor de Factor de Necrosis Tumoral tipo 1 (TNFR1), Receptor de Factor de Necrosis Tumoral tipo 2 (TNFR2), Receptor de Factor de Crecimiento Neural p75 (NGFR), el antígeno de superficie de linfocitos B CD40, el antígeno de linfocitos T OX-40, el receptor Apo-1 (también denominado Fas o CD95), el receptor Apo-3 (también denominado DR3, swl-1, TRAMP y LARD), el receptor denominado "Activador de Transmembrana e Interactor-CAML" o "TACI", proteína BCMA, DR4, DR5 (designadas alternativamente como Apo-2; TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 o KILLER), DR6, DcR1 (también mencionado como TRID, LIT o TRAIL-R3), DcR2 (también denominado TRAIL-R4 o TRUNDD), OPG, DcR3 (también denominado TR6 o M68), CAR1, HVEM (también denominado ATAR o TR2), GITR, ZTNFR-5, NTR-I, TNFL1, CD30, receptor β de linfotoxina (LTBr), receptor 4-IBB y TR9 (EP988, 371A1).

Los términos "ligando Apo-2", "Apo-2L", "Apo2L", "ligando Apo2L/TRAIL", y "TRAIL" se usan indistintamente en esta solicitud para referirse a una secuencia de polipéptidos que incluye residuos de aminoácidos 114-281, inclusive, 95-281, inclusive, residuos 92-281, inclusive, residuos 91-281, inclusive, residuos 41-281, inclusive, residuos 39-281, inclusive, residuos 15-281, inclusive, o residuos 1-281, inclusive, de la secuencia de aminoácidos mostrada en Figura 24 (SEQ ID No. 46), así como fragmentos biológicamente activos, variantes de deleción, inserción, o sustitución de las secuencias anteriores. En una incorporación, la secuencia polipeptídica comprende residuos 114-281 de Fig. 24 (SEQ ID No. 46). Opcionalmente, la secuencia polipeptídica comprende residuos

92-281 o residuos 91-281 de Figura 24 (SEQ ID No. 46). Los polipéptidos Apo-2L pueden codificarse por la secuencia nucleotídica natural mostrada en Figura 24 (SEQ ID No. 45). Opcionalmente, el codón que codifica el residuo Pro119 (Figura 24; (SEQ ID No. 45) puede ser "CCT" o "CCG". Opcionalmente, los fragmentos o variantes son biológicamente activos y tienen por lo menos alrededor de 80% de identidad de secuencia de aminoácidos, más preferiblemente por lo menos alrededor de 90% de identidad de secuencia, o por lo menos 95%, 96%, 97%, 98%, o 99% de identidad de secuencia con una cualquiera de las secuencias anteriores. La definición abarca variantes de sustitución del ligando Apo-2 en las cuales por lo menos uno de sus aminoácidos naturales se sustituyen por otro aminoácido tal como un residuo de alanina. La definición comprende asimismo un ligando Apo-2 de secuencia natural, aislado de una fuente de ligando Apo-2, o preparado por métodos recombinantes o sintéticos. El ligando Apo-2 de la invención incluye los polipéptidos designados como ligando Apo-2 o TRAIL divulgados en WO97/01633 publicada el 16 de enero de 1997, WO97/25428 publicada el 17 de julio de 1997, WO99/36535 publicada el 22 de julio de 1999, WO 01/00832 publicada el 4 de enero de 2001, WO02/09755 publicada el 7 de febrero de 2002, y WO 00/75191 publicada el 14 de diciembre de 2000 y Patente de los Estados Unidos No. 6,030,945 expedida el 29 de febrero de 2000. Los términos se utilizan para referirse generalmente a formas del ligando Apo-2, que incluyen formas monoméricas, diméricas, triméricas, hexaméricas o formas oligoméricas altas del polipéptido. Toda la numeración de residuos de aminoácidos mencionados en la secuencia Apo-2L usa la numeración de acuerdo con la Figura 24 (SEQ ID No. 46), a menos que se indique específicamente lo contrario.

"Receptor de ligando Apo-2" incluye los receptores mencionados en el arte como "DR4" y "DR5". Pan *et al.* han descrito el miembro de la familia de receptor TNF designado como "DR4" (Pan *et al., Science*, 276:111- 113 (1997); véase también WO98/32856 publicada el 30 de julio de 1998; WO 99/37684 publicada el 29 de julio de 1999; WO 00/73349

238

publicada el 7 de diciembre de 2000; US 6,433,147 expedida el 13 de agosto de 2002; US 6,461,823 expedida el 8 de octubre de 2002, y US 6,342,383 expedida el 29 de enero de 2002). Sheridan *et al., Science*, 277:818- 821 (1997) y Pan *et al., Science*, 277:815-818 (1997) describieron otro receptor para Apo2L/TRAIL (véase asimismo, WO98/51793 publicada el 19 de noviembre de 1998; WO98/41629 publicada el 24 de septiembre de 1998). Este receptor se designa como DR5 (el receptor se ha designado alternativamente como Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 o KILLER; Screaton *et al., Curr. Biol.*, 7:693-696 (1997); Walczak *et al., EMBO J.*, 16:5386-5387 (1997); Wu *et al., Nature Genetics*, 17:141-143 (1997); WO98/35986 publicada el 20 de agosto de 1998; EP870,827 publicada el 14 de octubre de 1998; WO98/46643 publicada el 22 de octubre de 1998; WO99/02653 publicada el 21 de enero de 1999; WO99/09165 publicada el 25 de febrero de 1999; WO99/11791 publicada el 11 de marzo de 1999; US 2002/0072091 publicada el 13 de agosto de 2002; US 2002/0098550 publicada el 7 2001; US 6,313,269 expedida el 6 de diciembre de 2001; US 2001/0010924 publicada el 2 de agosto de 2001; US 2003/01255540 publicada el 3 de julio de 2003; US 2002/0160446 publicada el 31 de octubre de 2002, US 2002/0048785 publicada el 25 de abril de 2002; US 6,569,642 expedida el 27 de mayo de 2003, US 6,072,047 expedida el 6 de junio de 2000, US 6,642,358 expedida el 4 de noviembre de 2003). Como se describe anteriormente, otros receptores para Apo-2L incluyen DcR1, DcR2, y OPG. El término "receptor Apo-2L", cuando se utiliza en esta solicitud, abarca un receptor de secuencia natural y variantes de receptor. Estos términos comprenden el receptor Apo-2L expresado en una variedad de mamíferos, incluidos humanos. El receptor Apo-2L puede expresarse endógenamente, ya que ocurre naturalmente en una variedad de linajes de tejido humano, o puede expresarse por métodos recombinantes o sintéticos. Un "receptor Apo-2L de secuencia natural" comprende un polipéptido que posee la misma secuencia de aminoácidos que un receptor Apo-2L, derivado de la naturaleza. Por consiguiente, un receptor Apo-2L de secuencia natural puede tener la secuencia de aminoácidos del receptor

Apo-2L de ocurrencia natural de cualquier mamífero, incluidos humanos. Tal receptor Apo-2L de secuencia natural puede aislarse de la naturaleza o puede producirse por medios recombinantes o sintéticos. El término "receptor Apo-2L de secuencia natural" abarca específicamente formas truncadas o secretadas, de ocurrencia natural, del receptor (*por ejemplo*, una forma soluble que contiene, por ejemplo, una secuencia de dominio extracelular), formas de variantes de ocurrencia natural (*por ejemplo*, formas alternativamente empalmadas) y variantes alélicas de ocurrencia natural. Variantes de receptor pueden incluir fragmentos o mutantes de deleción del receptor Apo-2L de secuencia natural. Figs. 25A-C muestran la secuencia de 411 aminoácidos de DR5 humano, junto con su secuencia nucleotídica (SEQ. ID. Nos. 47 y 48), como se publica en WO 98/51793 el 19 de noviembre de 1998. Una variante de empalme de transcripción de DR5 humano es conocida en el arte. Esta variante de empalme DR5 codifica la secuencia de 440 aminoácidos de DR5 humano, mostrada en Figs. 26A-C, junto con su secuencia nucleotídica (SEQ. ID. Nos. 49 y 50), y como se publica en WO 98/35986 el 20 de agosto de 1998.

"Anticuerpo de receptor de muerte" se usa en esta solicitud para referirse generalmente a un anticuerpo o anticuerpos dirigidos a un receptor en la superfamilia de receptores de factor de necrosis tumoral y que contienen un dominio de muerte capaz de señalar apoptosis, y tales anticuerpos incluyen el anticuerpo DR5 y el anticuerpo DR4.

"Anticuerpo de receptor DR5", "anticuerpo DR5", o "anticuerpo anti-DR5" se usan en un sentido amplio para referirse a anticuerpos que unen por lo menos una forma de un receptor DR5 o su dominio extracelular. Opcionalmente el anticuerpo DR5 se fusiona o enlaza a una secuencia heteróloga o molécula. Preferiblemente la secuencia heteróloga permite o ayuda al anticuerpo a formar complejos oligoméricos, o de orden más elevado. Opcionalmente, el anticuerpo DR5 se une al receptor DR5 pero no se une o reacciona cruzadamente con cualquier receptor Apo-2L adicional (por ejemplo, DR4, DcR1, o DcR2). Opcionalmente, el anticuerpo es un agonista de actividad de señalización de DR5.

Opcionalmente, el anticuerpo DR5 de la invención se une a un receptor DR5 en un rango de concentración de alrededor de 0.1 nM a alrededor de 20 mM, medido en un análisis de unión BIAcore. Opcionalmente, los anticuerpos DR5 de la invención exhiben un valor IC_{50} de alrededor de 0.6 nM a alrededor de 18 mM, medido en un análisis de unión BIAcore.

Puramente para los propósitos de esta solicitud, el término "Apomab" se refiere a un anticuerpo agonista que se une a DR5 y comprende las secuencias de aminoácidos ligeras variables y pesadas variables de SEQ ID Nos. 55 y 56. Preferiblemente Apomab comprende las cadenas ligeras y pesadas de SEQ ID Nos. 51 y 52, respectivamente.

II. Producción de Anticuerpos

Siguen a continuación técnicas para producir anticuerpos que pueden formularse de acuerdo con la presente invención.

(i) Selección y preparación de antígeno

Preferiblemente, el antígeno al cual se une el anticuerpo es una glucoproteína biológicamente importante, y la administración del anticuerpo a un mamífero que sufre de una enfermedad o trastorno puede resultar en un beneficio terapéutico en ese mamífero. No obstante, se contemplan asimismo anticuerpos dirigidos contra antígenos no polipeptídicos (tales como antígenos glucolipídicos asociados con tumores; véase La Patente de los Estados Unidos 5,091,178).

Donde el antígeno es un polipéptido, éste puede ser una molécula transmembrana (por ejemplo, receptor) o ligando tal como un factor de crecimiento. Antígenos ejemplares incluyen moléculas tales como renina; una somatotropina, incluidas la somatotropina humana y la somatotropina bovina; somatoliberina; paratirina; tirotropina; lipoproteínas; α-1-antitripsina; cadena A de la insulina; cadena B de la insulina; proinsulina; folitropina; calcitonina; lutropina; glucagón; factores de coagulación tales como factor VIIIC, factor IX, factor tisular (TF) y factor de von Willebrand; factores anticoagulantes tales como proteína C; factor natriurético atrial;

surfactante del pulmón; un activador de plasminógeno, tal como urocinasa o activador de plaminógeno tipo tisular (t-PA); bombesina; trombina; factor de crecimiento hematopoyético; factor-α y -β de necrosis tumoral; encefalinasa; RANTES (linfocito T regulado normalmente por activación, expresado y segregado); proteína inflamatoria de macrófago humano (MIP-1-α); albúmina sérica tal como albúmina sérica humana; sustancia inhibidora de Müller; cadena A de la relaxina; cadena B de la relaxina; prorelaxina; péptido asociado con la gonadotropina de ratón; una proteína microbiana, tal como betalactamasa; DNasa; IgE; un antígeno asociado con linfocitos T citotóxicos (CTLA), tal como CTLA-4; inhibina; activina; factor de crecimiento endotelial vascular (VEGF); receptores de hormonas o factores de crecimiento; proteína A o D; factores reumatoides; un factor neutrófico tal como factor neurotrófico derivado de hueso (BDNF), neurotrofina-3, -4, -5, ó -6 (NT-3, NT-4, NT-5, ó NT-6), o un factor de crecimiento neuronal tal como NGF-β; factor de crecimiento derivado de plaquetas (PDGF); factor de crecimiento de fibroblastos tal como aFGF y bFGF; factor de crecimiento epidérmico (EGF); factor de crecimiento transformante (TGF) tal como TGF-α y TGF-β, incluidos TGF-β1, TGF-β2, TGF-β3, TGF-β4, ó TGF-β5; un factor de necrosis tumoral (TNF) tal como TGF-α y TGF-β factor-I y -II de crecimiento insulinoide (IGF-I y IGF-II); des(1-3)-IGF-I (IGF-I del cerebro); proteínas de unión de factor insulinoide; proteínas CD tales como CD3, CD4, CD8, CD19 y CD20, CD22 y CD40; eritropoyetina; factores osteoinductores; inmunotoxinas; una proteína morfogenética de hueso (BMP); un interferón tal como interferón-α, -β, y -γ; factores estimulantes de colonias (CSFs), *por ejemplo,* M-CSF, GM-CSF, y G-CSF; interleucinas (IL), *por ejemplo,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 e IL-10; superóxido dismutasa; receptores de linfocitos T; proteínas de la membrana de superficie; factor acelerante de degradación (DAF); un antígeno vírico tal como, por ejemplo, una porción de la envoltura del SIDA; proteínas de transporte; receptores de direccionamiento (***"homing receptors"***); adresinas; proteínas reguladoras; integrinas tales como CD11a, CD11b, CD11c, CD18, una ICAM, VLA-4 y VCAM; un

antígeno asociado a tumor tal como receptor HER2, HER3 o HER4; y fragmentos de cualquiera de los polipéptidos ya enumerados.

Dianas moleculares ejemplares para anticuerpos comprendidos por la presente invención incluyen proteínas CD tales como CD3, CD4, CD8, CD19, CD20, CD22, CD34 y CD40; miembros de la familia de receptor ErbB tal como receptor EGF, receptor HER2, HER3 ó HER4; antígenos de superficie de linfocitos B, tales como CD20 o BR3, un miembro de la superfamilia de receptores de necrosis tumoral, incluidos DR5; antígeno de célula madre de próstata (PSCA); moléculas de adhesión celular tales como LFA-I, Macl, p150.95, VLA-4, ICAM-I, VCAM, integrina α4/ β7, e integrina αv/β3, incluidas sus subunidades α o β (por ejemplo, anticuerpos anti-CD11a, anti-CD18 o anti-CD11b); factores de crecimiento tales como VEGF, así como receptores para ellos; factor tisular (TF); un factor de necrosis tumoral (TNF) tal como TNF-α o TNF- β, interferón α (α-IFN); una interleucina, tal como IL-8; IgE; antígenos de grupo sanguíneo; receptor flk2/flt3; receptor de obesidad (OB); receptor mpl; CTLA-4; proteína C, etc.

Antígenos solubles o sus fragmentos, conjugados opcionalmente con otras moléculas, pueden utilizarse como inmunógenos para generar anticuerpos. Para moléculas transmembranales, tales como receptores, pueden usarse fragmentos de éstos (por ejemplo, el dominio extracelular de un receptor) como el inmunógeno. Alternativamente, células que expresan la molécula transmembranal pueden utilizarse como el inmunógeno. Tales células pueden derivarse de una fuente natural (por ejemplo, líneas de células cancerosas) o pueden ser células que se han transformado por técnicas recombinantes para expresar la molécula transmembranal. Otros antígenos y sus formas útiles para preparar anticuerpos serán evidentes a los expertos en el arte.

Para la producción de anticuerpos HER2, el antígeno HER2 a usarse para su producción puede ser, por ejemplo, una forma soluble del dominio extracelular de HER2 o su porción, que contenga el epítopo deseado. Alternativamente, células que expresan HER2 en su superficie celular (por

ejemplo, células NIH-3T3 transformadas para sobreexpresar HER2; o una línea de células de carcinoma tal como células SK-BR-3, véase Stancovski *et al., PNAS (USA)* 88:8691-8695 (1991)) pueden usarse para generar anticuerpos.

(ii) Anticuerpos monoclonales

Anticuerpos monoclonales se obtienen de una población de anticuerpos sustancialmente homogéneos, *es decir*, los anticuerpos individuales que comprenden la población son idénticos y/o se unen al mismo epítopo, excepto por posibles mutaciones que puedan surgir durante la producción del anticuerpo monoclonal. Por consiguiente, el calificativo "monoclonal" indica el carácter del anticuerpo como no siendo una mezcla de anticuerpos discretos.

Por ejemplo, los anticuerpos monoclonales pueden elaborarse usando el método del hibridoma, descrito por primera vez por Kohler *et al., Nature*, 256:495 (1975), o pueden elaborarse por métodos de ADN recombinante (Patente de los Estados Unidos No. 4,816,567).

En el método del hibridoma, se inmuniza un ratón u otro animal hospedero apropiado, como un hámster, en la forma descrita más arriba en el texto, para provocar linfocitos que produzcan o sean capaces de producir anticuerpos, que se unirán específicamente a la proteína usada para inmunización. Alternativamente, pueden inmunizarse linfocitos *in vitro*. En seguida, los linfocitos se fusionan con células de mieloma usando un agente fusionante apropiado, tal como polietilen glicol, para formar una célula de hibridoma (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

Las células de hibridoma así preparadas se siembran y cultivan en un medio de cultivo adecuado, que preferiblemente contiene una o más sustancias que inhiben el crecimiento o supervivencia de las células de mieloma progenitoras no fusionadas. Por ejemplo, si las células de mieloma progenitoras carecen de la enzima hipoxantina guanina fosforibosil transferasa (HGPRT o HPRT), el medio de cultivo para los hibridomas incluirá típicamente hipoxantina, aminopterina, y timidina

(medio HAT), sustancias que impiden el crecimiento de células deficientes en HGPRT.

Células preferidas de mieloma son aquellas que se fusionan eficientemente, favorecen una producción estable de alto nivel de anticuerpo por las células seleccionadas productoras de anticuerpo, y son sensibles a un medio tal como HAT. Entre éstas, se prefieren las líneas de mieloma murino, tales como las derivadas de tumores de ratón MOPC-21 y MPC-11 disponibles del Salk Institute Cell Distribution Center, San Diego, California USA, y células SP-2 (y sus derivados, por ejemplo, X63-Ag8-653) disponibles de la American Type Culture Collection, Rockville, Maryland, USA. También se han descrito líneas celulares de mieloma humano y heteromieloma ratón-humano para la producción de anticuerpos monoclonales humanos (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, páginas 51-63 (Marcel Dekker, Inc., New York, 1987)).

El medio de cultivo en el cual crecen las células del hibridoma se analiza para determinar la producción de anticuerpos monoclonales dirigidos contra el antígeno. Preferiblemente, la especificidad de unión de anticuerpos monoclonales producidos por células del hibridoma se determina por inmunoprecipitación o por un análisis de unión *in vitro*, tal como radioinmunoanálisis (RIA) o enzimoinmunoanálisis absorbente (ELISA).

Por ejemplo, la afinidad de unión del anticuerpo monoclonal puede determinarse mediante el análisis Scatchard de Munson *et al., Anal. Biochem.*, 107:220 (1980).

Una vez identificadas las células del hibridoma que producen anticuerpos de la especificidad, afinidad y/o actividad deseada, los clones pueden subclonarse limitando los procesos de dilución y cultivarse por métodos estándar (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986). Medios apropiados de cultivo para este propósito incluyen, por ejemplo, medio D-MEM o RPMI-1640.

Adicionalmente, las células del hibridoma pueden cultivarse *in vivo* como tumores ascíticos en un animal.

Los anticuerpos monoclonales segregados por los subclones se separan convenientemente del medio de cultivo, fluido ascítico, o suero mediante procedimientos convencionales de purificación de inmunoglobulina tales como, por ejemplo, proteína A-sefarosa, cromatografía de hidroxilapatita, electroforesis en gel, diálisis, o cromatografía de afinidad.

El ADN que codifica los anticuerpos monoclonales se aísla fácilmente y se secuencia usando procedimientos convencionales (*por ejemplo*, usando sondas de oligonucléotidos que son capaces de unirse específicamente a genes que codifican las cadenas pesadas y ligeras de anticuerpos murinos). Las células del hibridoma sirven como fuente preferida 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 simio, células derivadas de ovario de hámster chino (CHO), o células de mieloma, que no producen de otro modo proteína de anticuerpo, para obtener la síntesis de anticuerpos monoclonales en las células hospederas recombinantes. Artículos de reseña sobre la expresión recombinante en bacterias de ADN que codifica el anticuerpo incluyen Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) y Plückthun, *Immunol. Revs.* 130:151-188(1992).

En una incorporación adicional, los anticuerpos monoclonales o fragmentos de anticuerpo pueden aislarse de bibliotecas de bacteriófagos de anticuerpos, generadas usando las técnicas descritas en McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) y Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describen el aislamiento de anticuerpos humanos y murinos, respectivamente, usando bibliotecas de bacteriófagos. Publicaciones subsiguientes describen la producción de anticuerpos humanos de alta afinidad (margen nM) mediante entremezcla de cadenas (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), así como infección combinatoria y recombinación *in vivo* como una estrategia para

construir bibliotecas muy grandes de bacteriófagos (Waterhouse *et al., Nuc. Acids Res.*, 21:2265-2266 (1993)). Por consiguiente, estas técnicas son alternativas viables a las técnicas tradicionales de hibridoma de anticuerpo monoclonal para el aislamiento de anticuerpos monoclonales.

El ADN puede modificarse además, por ejemplo, sustituyendo la secuencia codificante para dominios constantes de cadenas ligeras y pesadas humanas en vez de las secuencias murinas homólogas (Patente de los Estados Unidos No. 4,816,567; Morrison *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), o empalmando covalentemente a la secuencia codificante de inmunoglobulina toda, o parte, de la secuencia codificante para un polipéptido distinto de inmunoglobulina.

Típicamente tales polipéptidos distintos de 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.

(iii) Anticuerpos humanizados

Métodos para humanizar anticuerpos no humanos se han descrito en el arte. Preferiblemente, un anticuerpo humanizado tiene uno o más residuos de aminoácidos introducidos en él de una fuente que es no humana. Estos residuos de aminoácidos no humanos se denominan a menudo residuos de "importación", los cuales se toman típicamente de un dominio variable de "importación". La humanización puede realizarse esencialmente siguiendo el método de Winter y colaboradores (Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-327 (1988); Verhoeyen *et al., Science* 239:1534-1536 (1988), o sustituyendo secuencias de región hipervariable por las secuencias correspondientes de un anticuerpo humano. En consecuencia, tales anticuerpos "humanizados" son anticuerpos quiméricos (Patente de los Estados Unidos No. 4,816,567), en donde sustancialmente menos de un dominio variable humano intacto se ha sustituido por la secuencia correspondiente de una especie no humana. En

la práctica, anticuerpos humanizados son típicamente anticuerpos humanos en los cuales algunos residuos de región hipervariable y, posiblemente, algunos residuos FR se sustituyen con residuos de sitios análogos en anticuerpos de roedor.

La elección de dominios variables humanos, ligeros y pesados, para usarlos en elaborar los anticuerpos humanizados es muy importante para reducir la antigenicidad. De acuerdo con el denominado método de "ajuste óptimo", se criba la secuencia del dominio variable de un anticuerpo de roedor contra toda la biblioteca de secuencias humanas de dominio variable conocidas. La secuencia humana más próxima a la del roedor se acepta entonces como la región de estructura alterna (FR) humana para el anticuerpo humanizado (Sims *et al., J. Immunol.*, 151:2296 (1993); Chothia *et al., J. Mol. Biol*, 196:901 (1987). Otro método usa una región de estructura alterna particular derivada de la secuencia consenso de todos los anticuerpos humanos de un subgrupo particular de cadenas ligeras y pesadas. La misma estructura alterna puede utilizarse para varios anticuerpos humanizados diferentes (Carter *et al., Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al., J. Immnol*, 151:2623 (1993)).

Es además importante que los anticuerpos se humanicen conservando una alta afinidad para el antígeno y otras propiedades biológicas favorables. Para lograr este objetivo, de acuerdo con un método preferido, anticuerpos humanizados se preparan por un proceso de análisis de las secuencias progenitoras y varios productos humanizados conceptuales usando modelos tri-dimensionales de las secuencias progenitoras y humanizadas. Comúnmente se cuenta con modelos tri-dimensionales de inmunoglobulina y son familiares a los expertos en el arte. Se dispone de programas de computador, los cuales ilustran y visualizan estructuras probables de conformación tri-dimensional de secuencias seleccionadas de la inmunoglobulina candidata. La inspección de estas visualizaciones permite el análisis del papel probable de los residuos en el funcionamiento de la secuencia de inmunoglobulina candidata, *es decir*, el análisis de residuos que influyen en la capacidad de la inmunoglulina candidata de unir su

antígeno. De esta forma, pueden seleccionarse residuos FR y combinarse de las secuencias del recipiendario y de importación, de modo que se obtenga la característica del anticuerpo deseada, tal como una creciente afinidad para el antígeno(s) diana. En general, los residuos de región hipervariable se hallan directa y, la mayoría de las veces, sustancialmente implicados en influir en la unión del antígeno.

WO01/00245 describe la producción de anticuerpos HER2 humanizados ejemplares, que unen HER2 y bloquean la activación de ligando de un receptor HER. El anticuerpo humanizado de interés particular en esta solicitud bloquea la activación de MAPK mediada por EGF, TGF-α y/o HRG, esencialmente en forma tan efectiva como el anticuerpo monoclonal murino 2C4 (o su fragmento Fab) y/o une HER2 esencialmente en forma tan efectiva como el anticuerpo monoclonal murino 2C4 (o su fragmento Fab). El anticuerpo humanizado en esta solicitud puede, por ejemplo, comprender residuos no humanos de región hipervariable, incorporados en un dominio pesado variable humano, y puede comprender además una sustitución de región de estructura alterna (FR) en una posición seleccionada del grupo conformado por 69H, 71H y 73H, utilizando el sistema de numeración de dominio variable expuesto 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 o todas las posiciones 69H, 71H y 73H.

Un anticuerpo humanizado ejemplar de interés en esta solicitud comprende residuos determinantes de complementariedad de dominio pesado variable GFTFTDYTMX, donde X es preferiblemente D o S (SEQ ID No. 7); DVNPNSGGSIYNQRFKG (SEQ ID NO. 8); y/o NLGPSFYFDY (SEQ ID No. 9), comprendiendo opcionalmente modificaciones de aminoácidos de aquellos residuos CDR, por ejemplo, donde las modificaciones esencialmente mantienen o mejoran la afinidad del anticuerpo. Por ejemplo, la variante del anticuerpo de interés puede tener desde alrededor de uno a alrededor de siete o alrededor de cinco

sustituciones de aminoácido en las anteriores secuencias CDR pesadas variables. Tales variantes de anticuerpo pueden prepararse mediante maduración por afinidad, por ejemplo, como se describe más adelante. El anticuerpo humanizado más preferido comprende la secuencia de aminoácidos de dominio pesado variable en SEQ ID No. 4.

El anticuerpo humanizado puede comprender residuos determinantes de complementariedad de dominio ligero variable KASQDVSIGVA (SEQ ID NO. 10); SASYXXX, donde la X como posición 5 es preferiblemente R o L, en donde la X en la posición 6 es preferiblemente Y o E, y la X como posición 7 es preferiblemente T o S (SEQ ID No. 11); y/o QQYYIYPYT (SEQ ID No. 12), por ejemplo, además de aquellos residuos CDR de dominio pesado variable en el párrafo precedente. Tales anticuerpos humanizados comprenden opcionalmente modificaciones de aminoácidos de los residuos CDR anteriores, por ejemplo donde las modificaciones mantienen o mejoran esencialmente la afinidad del anticuerpo. Por ejemplo, la variante del anticuerpo de interés puede tener desde alrededor de una a alrededor de siete, o alrededor de cinco sustituciones de aminoácidos en las secuencias CDR ligeras variables ya mencionadas. Tales variantes de anticuerpo pueden prepararse mediante afinidad por maduración, por ejemplo, como se describe a continuación. El anticuerpo humanizado más preferido comprende la secuencia de aminoácidos de dominio ligero variable en SEQ ID No. 3.

La presente solicitud contempla igualmente anticuerpos madurados por afinidad, los cuales unen HER2 y bloquean la activación de ligando de un receptor HER. El anticuerpo progenitor puede ser un anticuerpo humano o un anticuerpo humanizado, por ejemplo, uno que comprenda las secuencias pesadas y/o ligeras variables de SEQ ID Nos. 3 y 4, respectivamente (es decir, variante 574). El anticuerpo madurado por afinidad preferiblemente se une al receptor HER2 con una afinidad superior a la del 2C4 murino o variante 574 (por ejemplo, desde alrededor de dos o alrededor de cuatro veces, a alrededor de 100 veces o alrededor de 1.000 veces la afinidad mejorada, por ejemplo, como se evalúa usando un

250

dominio extracelular (ECD) HER2 (ELISA). Residuos CDR pesados variables ejemplares para substitución incluyen H28, H30, H34, H35, H64, H96, H99, o combinaciones de dos o más (por ejemplo, dos, tres, cuatro, cinco, seis, o siete de estos residuos). Ejemplos de residuos CDR ligeros variables para alteración incluyen L28, L50, L53, L56, L91, L92, L93, L94, L96, L97 o combinaciones de dos o más (por ejemplo, dos a tres, cuatro, cinco o hasta a alrededor de diez de estos residuos). Se contemplan varias formas del anticuerpo humanizado o anticuerpo madurado por afinidad. Por ejemplo, el anticuerpo humanizado o anticuerpo madurado por afinidad puede ser un fragmento de anticuerpo, tal como un Fab, que se conjuga opcionalmente con uno o más agentes citotóxicos, con el fin de generar un inmunoconjugado. Alternativamente, el anticuerpo humanizado o el anticuerpo madurado por afinidad puede ser un anticuerpo de longitud total, tal como un anticuerpo IgG1 de longitud total.

(iv) Anticuerpos humanos

Como una alternativa a la humanización, pueden generarse anticuerpos humanos. Por ejemplo, actualmente es posible producir animales transgénicos (*verbigracia*, ratones) que, luego de la inmunización, son capaces de producir un completo repertorio de anticuerpos humanos en la ausencia de producción de inmunoglobulina endógena. Por ejemplo, se ha descrito que la deleción homocigótica del gen de la región de unión a la cadena pesada (J_H) del anticuerpo en ratones mutantes quiméricos y de línea germinativa resulta en la completa inhibición de la producción endógena de anticuerpos. La transferencia del arreglo de genes de inmunoglobulina de línea germinativa humana en tales ratones mutantes de línea germinativa resultará en la producción de anticuerpos humanos a la exposición al antígeno. Véase, *por ejemplo*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993); Patentes de los Estados Unidos No. 5,591,669, 5,589,369 y 5,545,807.

Alternativamente, puede usarse tecnología exposición en bacteriófagos para producir anticuerpos humanos y fragmentos de

anticuerpo *in vitro*, a partir de repertorios de genes de dominio variable (V) de inmunoglobulina de donantes no inmunizados. De acuerdo con esta técnica, genes de dominio V de anticuerpo se clonan sin cambio en la fase de lectura (***"are cloned in-frame"***) en un gen de cápside mayor o menor de un bacteriófago filamentoso, tal como M13 ó fd, y se despliegan como fragmentos funcionales de anticuerpo en la superficie de la partícula del bacteriófago. Debido a que la partícula filamentosa contiene una copia de ADN monocatenario del genoma del bacteriófago, las selecciones basadas en las propiedades funcionales del anticuerpo resultan igualmente en la selección del gen codificante del anticuerpo que exhibe estas propiedades. Por consiguiente, el bacteriófago imita algunas de las propiedades del linfocito B. La exposición en bacteriófagos puede realizarse en una variedad de formatos; para su reseña véase, *por ejemplo*, Johnson, Kevin S. y Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Para la exposición en bacteriófagos pueden emplearse varias fuentes de segmentos de gen V. Clackson *et al., Nature* 352:624-628 (1991) aislaron un arreglo diverso de anticuerpos de anti-oxazolona de una pequeña biblioteca combinatoria aleatoria de genes V, derivados de los bazos de ratones inmunizados. Puede construirse un repertorio de genes V de donantes no inmunizados humanos, y anticuerpos a un arreglo diverso de antígenos (incluidos auto-antígenos) pueden aislarse siguiendo esencialmente las técnicas descritas por Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), o Griffith *et al., EMBO J.* 12:725-734 (1993). Véase también las Patentes de los Estados Unidos No. 5,565,332 y 5,573,905.

Como ya se discutió, pueden generarse asimismo anticuerpos humanos mediante linfocitos B activados *in vitro* (véase las Patentes de los Estados Unidos 5,567,610 y 5,229,275).

Anticuerpos HER2 humanos se describen en la Patente de los Estados Unidos No. 5,772,997 expedida el 30 de junio de 1998 y WO 97/00271 publicada el 3 de enero de 1997.

(v) Fragmentos de anticuerpo

Se han desarrollado varias técnicas para la producción de fragmentos de anticuerpo. Tradicionalmente, estos fragmentos se derivaron por digestión proteolítica de anticuerpos intactos (véase, *por ejemplo*, Morimoto *et al., Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); y Brennan *et al., Science 229*:81 (1985)). Sin embargo, estos fragmentos pueden producirse ahora directamente por células hospederas recombinantes. Por ejemplo, los fragmentos de anticuerpo pueden aislarse de las bibliotecas de bacteriófagos de anticuerpos, discutidas anteriormente. Alternativamente, fragmentos Fab'-SH pueden recuperarse directamente de *E. coli* y acoplarse químicamente para formar fragmentos $F(ab')_2$ (Carter *et al., Bio/Technology* 10:163-167 (1992)). De acuerdo con otra metodología, fragmentos $F(ab')_2$ pueden aislarse directamente de cultivo de células hospederas recombinantes. Otras técnicas para la producción de fragmentos de anticuerpo serán evidentes al profesional experto. En otras incorporaciones, el anticuerpo de elección es un fragmento Fv de cadena única (scFv). Véase WO 93/16185; Patente de los Estados Unidos No. 5,571,894 y Patente de los Estados Unidos No. 5,587,458. El fragmento de anticuerpo puede ser igualmente un "anticuerpo lineal", *por ejemplo*, como se describe en la Patente de los Estados Unidos 5,641,870. Tales fragmentos de anticuerpo lineal pueden ser monoespecíficos o bi-específicos.

(vi) Anticuerpos biespecíficos

Anticuerpos biespecíficos son anticuerpos que tienen especificidades de unión para por lo menos dos epítopos diferentes. Anticuerpos biespecíficos ejemplares pueden unirse a dos epítopos diferentes de la proteína HER2. Otros anticuerpos semejantes pueden combinar un sitio de unión HER2 con sitio(s) de unión para EGFR, HER3 y/o HER4. Alternativamente, un brazo HER2 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 celular a la célula que expresa HER2. Anticuerpos biespecíficos pueden usarse asimismo para localizar agentes citotóxicos contra células que expresan HER2. Estos anticuerpos poseen un brazo de unión HER2 y un brazo que une el agente citotóxico (por ejemplo, saporina, anti-interferón-α, vinca alcaloide, cadena de ricina A, metotrexato o el isótopo radioactivo hapteno). Los anticuerpos biespecíficos pueden prepararse como anticuerpos de longitud total o fragmentos de anticuerpo (por ejemplo, anticuerpos biespecíficos $F(ab')_2$).

WO 96/16673 describe un anticuerpo HER2/FcγRIH biespecífico, y la Patente de los Estados Unidos No. 5,837,234 divulga un anticuerpo HER2/FcγRI biespecífico IDM1 (Osidem). Un anticuerpo HER2/Fcα biespecífico se muestra en WO98/02463. La Patente de los Estados Unidos No. 5,821,337 enseña una anticuerpo HER2/CD3 biespecífico. MDX-210 es un HER2-FcγRIII Ab biespecífico.

Métodos para elaborar anticuerpos biespecíficos son conocidos en el arte. La producción tradicional de anticuerpos biespecíficos de longitud total se basa en la co-expresión de dos parejas de cadena ligera-cadena pesada de inmunoglobulina, donde las dos cadenas tienen especificidades diferentes (Millstein *et al.*, *Nature*, 305:537-539 (1983). Debido a la clasificación aleatoria de cadenas ligeras y pesadas de inmunoglobulina, estos hibridomas (cuadromas) producen una mezcla potencial de 10 diferentes moléculas de anticuerpo, de las cuales sólo una posee la estructura biespecífica correcta. La purificación de la molécula correcta, lo cual se hace usualmente por los pasos de cromatografía de afinidad, es más bien difícil, y los rendimientos del producto son bajos. Procedimientos similares se divulgan en WO 93/08829, y en Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

De acuerdo con un enfoque diferente, dominios variables de anticuerpo con las especificidades de unión deseadas (sitios combinadores de anticuerpo-antígeno) se fusionan a secuencias de dominio constante de inmunoglobulina. La fusión es preferiblemente con un dominio constante de cadena pesada de inmunoglobulina, que comprende por lo menos parte

de las regiones de unión flexible, CH2 y CH3. Se prefiere que la primera región constante de cadena pesada (CH1) contenga el sitio necesario para unión de la cadena ligera, presente en por lo menos una de las fusiones. Los ADN que codifican las fusiones de cadena pesada de inmunoglobulina y, si se desea, la cadena ligera de inmunoglobulina, se insertan en factores de expresión separados, y se co-transfectan en un organismo hospedero apropiado. Esto facilita una gran flexibilidad en ajustar las proporciones mutuas de los tres fragmentos del polipéptido en incorporaciones, cuando proporciones desiguales de las tres cadenas polipeptídicas usadas en la construcción proporcionan los rendimientos óptimos. No obstante, es posible insertar las secuencias codificantes para las dos o todas las tres cadenas polipeptídicas en un vector de expresión, cuando la expresión de por lo menos dos cadenas polipeptídicas en proporciones iguales resulta en altos rendimientos, o cuando las proporciones no son de significación particular.

En una incorporación preferida de este enfoque, los anticuerpos biespecíficos se componen de una cadena pesada de inmunoglobulina híbrida con una primera especificidad de unión en un brazo, y una pareja de cadena ligera-cadena pesada de inmunoglobulina híbrida (que proporciona una segunda especificidad de unión) en el otro brazo. Se encontró que esta estructura asimétrica facilita la separación del compuesto biespecífico deseado de combinaciones no deseadas de cadena de inmunoglobulina, en la medida en que la presencia de una cadena ligera de inmunoglobulina en sólo una mitad de las moléculas biespecíficas ofrece un modo fácil de separación. Esta metodología se divulga en WO 94/04690. Para mayores detalles de generar anticuerpos biespecíficos, véase, por ejemplo, Suresh *et al., Methods in Enzymology* 121: 210 (1986).

De acuerdo con otro enfoque descrito en la Patente de los Estados Unidos 5,731,168, la interfase entre una pareja de moléculas de anticuerpo puede manipularse para maximizar el porcentaje de heterodímeros que se recuperan del cultivo de células recombinantes. La interfase preferida comprende por lo menos una parte del dominio C_H3 de un dominio

constante de anticuerpo. En este método, una o más cadenas laterales pequeñas de aminoácidos de la interfase de la primera molécula de anticuerpo se reemplazan con cadenas laterales más grandes (*por ejemplo,* tirosina o triptófano). "Cavidades" compensatorias de tamaño idéntico o similar a la cadena(s) lateral(es) grande(s) se crea(n) en la interfase de la segunda molécula de anticuerpo, reemplazando cadenas laterales de aminoácidos grandes con más pequeñas (*por ejemplo,* alanina o treonina). Esto proporciona un mecanismo para aumentar el rendimiento del heterodímero sobre otros productos finales indeseados tales como homodímeros.

Anticuerpos biespecíficos incluyen anticuerpos enlazados cruzadamente o de "heteroconjugado". Por ejemplo, uno de los anticuerpos en el heteroconjugado puede acoplarse a avidina, el otro a biotina. Tales anticuerpos se han propuesto, por ejemplo, para tomar como diana células del sistema inmunitario contra células no deseadas (Patente de los Estados Unidos No. 4,676,980), y para tratamiento de la infección por VIH (WO 91/00360, WO 92/200373, y EP 03089). Los anticuerpos de heteroconjugado pueden elaborarse usando cualesquiera métodos convenientes de enlazamiento cruzado. Agentes apropiados de enlazamiento cruzado son bien conocidos en el arte, y se divulgan en la Patente de los Estados Unidos No. 4,676,980, junto con un número de técnicas de enlazamiento cruzado.

En la literatura se han descrito técnicas para generar anticuerpos biespecíficos de fragmentos de anticuerpo. Por ejemplo, anticuerpos biespecíficos pueden prepararse usando enlazamiento químico. Brennan *et al., Science* 229: 81 (1985) describen un procedimiento en donde anticuerpos de longitud total se segmentan proteolíticamente para generar fragmentos $F(ab')_2$. Estos fragmentos se reducen en la presencia del agente acomplejante ditiol arsenita de sodio para estabilizar ditioles vecinos e impedir la formación de disulfuro intermolecular. Los fragmentos Fab' generados se convierten luego en derivados de tionitrobenzoato (TNB). Uno de los derivados Fab'-TNB se reconvierte entonces en el derivado Fab'-

tiol por reducción con mercaptoetilamina y se mezcla con una cantidad equimolar el otro derivado Fab'-TNB para formar el anticuerpo biespecífico. Los anticuerpos biespecíficos producidos pueden usarse como agentes para la inmovilización selectiva de enzimas.

El avance reciente ha facilitado la recuperación directa de fragmentos Fab'-SH de *E. coli*, los cuales pueden acoplarse químicamente para formar anticuerpos biespecíficos. Shalaby *et al., J. Exp. Med.*, 175: 217-225 (1992) describen la producción de una molécula de anticuerpo biespecífico totalmente humanizado $F(ab')_2$. Cada fragmento Fab' se segregó separadamente de *E. coli* y se sometió a acoplamiento químico dirigido *in vitro* para formar el anticuerpo biespecífico. El anticuerpo biespecífico así formado fue capaz de unirse a células que sobreexpresaban el receptor HER2 y linfocitos T humanos normales, así como desencadenar la actividad lítica de linfocitos citotóxicos humanos contra dianas de tumor de mama humano. Se han descrito asimismo varias técnicas para elaborar y aislar fragmentos de anticuerpo biespecífico, directamente del cultivo de células recombinantes. Por ejemplo, se han producido anticuerpos biespecíficos usando cremalleras de leucina. Kostelny *et al., J. Immunol.*, 148(5): 1547-1553 (1992). Los péptidos de cremallera de leucina de las proteínas Fos y Jun se enlazaron a las porciones Fab' de dos anticuerpos diferentes mediante fusión de genes. Los homodímeros de anticuerpo se redujeron en la región de unión flexible para formar monómeros y luego se re-oxidaron para formar los heterodímeros del anticuerpo. Este método puede utilizarse asimismo para la producción de homodímeros de anticuerpo. La tecnología "diacuerpo" descrita por Hollinger *et al., Proc. Natl. Acad Sci. USA*, 90: 6444-6448 (1993) ha proporcionado un mecanismo alternativo para elaborar fragmentos de anticuerpos biespecíficos. Los fragmentos comprenden un dominio variable de cadena pesada (V_H), conectado a un dominio variable de cadena ligera (V_L) por un conector que es demasiado corto para permitir el emparejamiento entre los dos dominios en la misma cadena. Por consiguiente, los dominios V_H y V_L de un fragmento son forzados a emparejarse con los dominios V_L y V_H complementarios del otro

fragmento, formando así dos sitios de unión a antígeno. En la misma forma se ha informado de otra estrategia para elaborar fragmentos de anticuerpos biespecíficos mediante el uso de dímeros Fv de cadena única (sFv). Véase Gruber *et al., J. Immunol.*, 152:5368 (1994).

Se ha divulgado igualmente otra estrategia para elaborar fragmentos de anticuerpo biespecífico mediante el uso de dímeros Fv de cadena única (sFv). Véase Gruber *et al., J. Immunol,* 152:5368 (1994).

Se contemplan anticuerpos con más de dos valencias. Por ejemplo, pueden prepararse anticuerpos triespecíficos. Tutt *y colaboradores, J. Immunol.* 147: 60 (1991).

(vii) Otras modificaciones de secuencias de aminoácidos

Se contemplan modificaciones de secuencias de aminoácidos de los anticuerpos descritos en esta solicitud. Por ejemplo, puede ser deseable mejorar la afinidad de unión y/u otras propiedades biológicas del anticuerpo. Variantes de la secuencia de aminoácidos del anticuerpo se preparan introduciendo cambios nucleotídicos apropiados en el ácido nucleico del anticuerpo, o por síntesis peptídica. Tales modificaciones incluyen, por ejemplo, deleciones de, y/o inserciones en y/o sustituciones de, residuos en las secuencias de aminoácidos del anticuerpo. Cualquier combinación de deleción, inserción, y sustitución se realiza para arribar al constructo final, siempre que el constructo final posea las características deseadas. Los cambios de aminoácidos pueden alterar igualmente el proceso pos-translacional del anticuerpo, tal como modificando el número o posición de los sitios de glucosilación.

Un método útil para la identificación de ciertos residuos o regiones del anticuerpo que son localizaciones preferidas para mutagénesis se denomina "mutagénesis por rastreo de alanina" como se describe por Cunningham and Wells Science, 244:1081-1085 (1989). Aquí, se identifican un residuo 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 (muy preferiblemente alanina o polialanina) para afectar la interacción de los aminoácidos con el antígeno.

Aquellas localizaciones de aminoácidos que demuestren sensibilidad funcional a las sustituciones se refinan entonces introduciendo variantes adicionales u otras en, o para, los sitios de substitución. En consecuencia, aunque el sitio para introducir una variación en la secuencia de aminoácidos está predeterminado, la naturaleza de la mutación *per se* no necesita estar predeterminada. Por ejemplo, para analizar el desempeño de una mutación en un sitio dado, se conduce mutagénesis aleatoria o por rastreo de alanina en el codón o región diana, y se criban las variantes del anticuerpo expresadas con respecto a la actividad deseada.

Las inserciones de secuencias de aminoácidos incluyen fusiones amino- y/o carboxilo-terminales que van en longitud desde un residuo a polipéptidos que contienen cien o más residuos, así como inserciones intrasecuencia de residuos simples o múltiples de aminoácidos. Ejemplos de inserciones terminales incluyen un anticuerpo con un residuo metionilo N-terminal o el anticuerpo fusionado a un polipéptido citotóxico. Otras variantes de inserción de la molécula de anticuerpo incluyen la fusión al N- o C-término del anticuerpo a una enzima (por ejemplo, para ADEPT) o un polipéptido que aumenta la semivida sérica del anticuerpo.

Otro tipo de variante es una variante de sustitución de aminoácidos. Estas variantes tienen por lo menos un residuo de aminoácidos en la molécula de anticuerpo reemplazado por un residuo diferente. Los sitios de máximo interés para mutagénesis de sustitución incluyen las regiones hipervariables, pero pueden contemplarse asimismo alteraciones de la región FR o Fc. Sustituciones conservadoras se muestran en la Tabla 1 bajo el encabezamiento de "sustituciones preferidas". Si tales sustituciones resultan en un cambio en la actividad biológica, entonces pueden introducirse cambios más sustanciales, denominados "sustituciones ejemplares" en la Tabla 1, o como se describe con más detalle a continuación con referencia a clases de aminoácidos, y cribarse los productos.

Tabla 1

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

Modificaciones sustanciales en las propiedades biológicas del anticuerpo se realizan seleccionando sustituciones que difieren significativamente en su efecto sobre mantener (a) la estructura polipeptídica en el área de la sustitución, por ejemplo, como una conformación de hoja o conformación helicoidal, (b) la carga de hidrofobicidad de la molécula en el sitio diana, o (c) el volumen de la cadena lateral. Los aminoácidos pueden agruparse de acuerdo con las

similitudes en las propiedades de sus cadenas laterales (en A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) no polares: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) polares no cargados: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) ácidos: Asp (D), Glu (E)

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

Alternativamente, residuos de ocurrencia natural pueden dividirse en grupos, basados en propiedades comunes de cadena lateral:

(1) hidrófobos: Norleucina, Met, Ala, Val, Leu, Ile;

(2) hidrófilos neutros: Cys, Ser, Thr, Asn, Gln;

(3) ácidos: Asp, Glu;

(4) básicos: His, Lys, Arg;

(5) residuos que influyen en la orientación de la cadena: Gly, Pro;

(6) aromáticos: 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 su estabilidad (particularmente donde el anticuerpo es un fragmento de anticuerpo tal como un fragmento Fv).

Un tipo particularmente preferido de variante de sustitución involucra sustituir uno o más residuos de región hipervariable de un anticuerpo progenitor (*por ejemplo*, un anticuerpo humano o humanizado). Generalmente, la variante(s) resultante para el desarrollo posterior tendrá

propiedades biológicas mejoradas relativas al anticuerpo progenitor, del cual se generan. Un modo conveniente para generar tales variantes de sustitución es maduración por afinidad usando la técnica de exposición en bacteriófagos. En síntesis, varios sitios de región hipervariable (*por ejemplo*, 6-7 sitios) se mutan para generar todas las sustituciones posibles de aminoácidos en cada sitio. Las variantes de anticuerpo así generadas se exponen en una manera monovalente de partículas de bacteriófago filamentoso como fusiones al producto del gen III de M13 empaquetado con cada partícula. Las variantes expuestas en bactriófago se criban entonces con respecto a su actividad biológica (*por ejemplo*, afinidad de unión), tal como se divulga en esta solicitud. Con el fin de identificar sitios candidatos de región hipervariable para modificación, puede realizarse mutagénesis dirigida de rastreo de alanina, para identificar residuos de región hipervariable que contribuyen significativamente a la unión a antígeno. Alternativa, o adicionalmente, puede ser benéfico analizar una estructura cristalina del complejo antígeno-anticuerpo para identificar puntos de contacto entre el anticuerpo y el antígeno. Tales residuos de contacto y residuos contiguos son candidatos para sustitución de acuerdo con las técnicas elaboradas en esta solicitud. Una vez se generan tales variantes, el panel de variantes se somete a cribado, como se describe en esta solicitud, y pueden seleccionarse para desarrollo posterior anticuerpos con propiedades superiores en uno o más análisis relevantes.

Otro tipo de variante de aminoácidos del anticuerpo altera el patrón de glucosilación original del anticuerpo. Por alterar se da a entender eliminar una o más fracciones carbohidrato halladas en el anticuerpo, y/o agregar uno o más sitios de glucosilación que no están presentes en el anticuerpo.

La glucosilación de polipéptidos es típicamente N- u O-enlazada. N-enlazada se refiere a la unión de la fracción carbohidrato a la cadena lateral de un residuo de asparagina. Las secuencias tripeptídicas asparagina-X-serina y asparagina-X-treonina, donde X es cualquier aminoácido excepto prolina, son las secuencias de reconocimiento para unión enzimática de la

fracción carbohidrato a la cadena lateral de asparagina. Por consiguiente, la presencia de cualquiera de estas secuencias tripeptídicas en un polipéptido crea un sitio potencial de glucosilación. Glucosilación O- enlazada se refiere a la unión de uno de los azúcares N-acetilgalactosamina, galactosa, o xilosa a un hidroxiaminoácido, muy comúnmente serina o treonina, si bien pueden usarse igualmente 5-hidroxiprolina o 5-hidroxilisina.

La adición de sitios de glucosilación al anticuerpo se lleva convenientemente a cabo alterando la secuencia de aminoácidos, de manera que contenga una o más de las secuencias tripeptídicas ya descritas (para sitios de glucosilación N-enlazados). La alteración puede hacerse además por la adición de, o sustitución por, uno o más residuos de serina o treonina a la secuencia del anticuerpo original (para sitios de glucosilación O-enlazados).

Donde el anticuerpo comprende una región Fc, el carbohidrato unido a ella puede alterarse. Por ejemplo, anticuerpos con una estructura carbohidrato madura, que carece de la fucosa unida a una región Fc del anticuerpo, se describen en la Solicitud de Patente de los Estados Unidos No. US 2003/0157108 Al, Presta, L. Véase también US 2004/0093621 Al (Kyowa Hakko Kogyo Co., Ltd). Anticuerpos con N-acetilglucosamina bisectante (GlcNAc) en el carbohidrato unido a una región Fc del anticuerpo se referencian en WO03/011878, Jean-Mairet *et al.*, y la Patente de los Estados Unidos No. 6,602,684, Umana *et al.* Anticuerpos con por lo menos un residuo de galactosa en el oligosacárido unido a una región Fc del anticuerpo se divulgan en WO97/30087, Patel *et al.* Véase, además, WO98/58964 (Raju, S.) y WO99/22764 (Raju, S.) en lo concerniente a anticuerpos con carbohidrato alterado unido a la región Fc de los mismos. Composiciones de anticuerpo, que comprenden especies principales del anticuerpo con tales estructuras carbohidrato unidas a la región Fc, se contemplan en esta solicitud.

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.

(viii) Cribado para detectar anticuerpo con las propiedades deseadas

Ya se describieron técnicas para generar anticuerpos. Se pueden además seleccionar anticuerpos con ciertas características biológicas, según se desee.

Para identificar un anticuerpo que bloquea la activación de ligando de un HER, puede determinarse la capacidad del anticuerpo de bloquear la unión de ligando HER a células que expresan el receptor HER (*por ejemplo*, en conjugación con otro receptor HER, con el cual el receptor HER de interés forma un hetero-oligómero HER). Por ejemplo, células, que sobreexpresan naturalmente, o transfectadas para expresar, receptores HER del hetero-oligómero HER pueden incubarse con el anticuerpo y luego exponerse al ligando HER marcado. Entonces puede evaluarse la capacidad del anticuerpo HER de bloquear la unión de ligando al receptor HER en el hetero-oligómero HER.

Por ejemplo, la inhibición de la unión de HRG a líneas de células de tumor de mama MCF7 por anticuerpos HER2 puede realizarse usando cultivos monocapa MCF7 sobre hielo en un formato de placa de 24 pocillos, esencialmente como se describe en WO01/00245. Anticuerpos monoclonales HER2 pueden agregarse a cada pocillo e incubarse por 30 minutos. A continuación puede agregarse rHRGβ$1_{177-224}$ marcado con ^{125}I (25 pm), 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. En una incorporación, el anticuerpo que bloquea la activación de ligando de un receptor HER tendrá un IC_{50} para inhibir la unión de HRG a células MCF7 en este análisis de alrededor de 50nM o menos, más preferiblemente 10nM o menos. Donde el anticuerpo es un

fragmento de anticuerpo tal como un fragmento Fab, el IC_{50} para inhibir la unión de HRG a células MCF7 en este análisis puede ser, por ejemplo, de alrededor de 100nM o menos, más preferiblemente 50nM o menos.

Alternativa, o adicionalmente, puede evaluarse la capacidad del anticuerpo HER de bloquear la fosforilación de tirosina estimulada por ligando HER de un receptor HER presente en un hetero-oligómero HER. Por ejemplo, células que expresan endógenamente los receptores HER o transfectadas para expresarlos, pueden incubarse con el anticuerpo y luego analizarse con respecto a la actividad de fosforilación de tirosina dependiente de ligando HER, usando un anticuerpo monoclonal anti-fosfotirosina (que se conjuga opcionalmente con un marcador detectable). El análisis de activación de receptor de cinasa descrito en la Patente de los Estados Unidos 5,766,863 es también utilizable para determinar la activación del receptor HER y el bloqueo de esa actividad por el anticuerpo.

En una incorporación, se puede cribar un anticuerpo que inhiba la estimulación HRG de fosforilación de tirosina p180 en células MCF7, esencialmente como se describe en WO01/00245. Las células MCF7 pueden esparcirse en placas de 24 pocillos, y pueden agregarse anticuerpos monoclonales contra HER2 a cada pocillo e incubarse durante 30 minutos a temperatura de la sala; luego puede agregarse $rHRG\beta_{177-244}$ a cada pocillo a una concentración final de 0.2 nM, y la incubación puede continuarse durante 8 minutos. Puede aspirarse el medio de cada pocillo, y las reacciones pueden detenerse por la adición de 100 µl de amortiguador 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 banda reactiva predominante a $M_r \sim 180.000$ mediante densitometría de reflexión. El anticuerpo seleccionado inhibirá de forma preferiblemente significativa la estimulación HRG de la fosforilación de tirosina p180 a alrededor de 0-35% del control en este análisis. Puede prepararse una curva de respuesta a

la dosis para la estimulación HRG de la fosforilación de la tirosina p180, determinada por densitometría de reflexión, y calcularse un IC_{50} para el anticuerpo de interés. En una incorporación, el anticuerpo que bloquea la activación de ligando de un receptor HER tendrá un IC_{50} para inhibir la estimulación HRG de la fosforilación de la tirosina p180 en este análisis de alrededor de 50nM o menos, más preferiblemente 10nM o menos. Donde el anticuerpo es un fragmento de anticuerpo tal como un fragmento Fab, el IC_{50} para inhibir la estimulación HRG de la fosforilación de la tirosina p180 en este análisis puede ser, por ejemplo, de alrededor de 100nM o menos, más preferiblemente 50nM o menos.

Se pueden evaluar también los efectos inhibidores de crecimiento de un anticuerpo en células MDA-MB-175, por ejemplo, esencialmente como se describe en Schaefer *et al.*, *Oncogene* 15:1385-1394 (1997)). De acuerdo con este análisis, células MDA-MB-175 pueden tratarse con un anticuerpo monoclonal HER2 (10 μg/mL) durante 4 días y teñirse con cristal violeta. La incubación con un anticuerpo HER2 puede mostrar un efecto inhibidor de crecimiento, en esta línea celular, similar al desplegado por el anticuerpo monoclonal 2C4. En una incorporación adicional, HRG exógena no reversará significativamente esta inhibición. Preferiblemente, el anticuerpo será capaz de inhibir la proliferación celular de las células MDA-MB-175 en una medida mayor que el anticuerpo monoclonal 4D5 (y, opcionalmente, en una medida mayor que el anticuerpo monoclonal 7F3), ambos en la presencia y ausencia de una HRG exógena.

En una incorporación, el anticuerpo HER2 de interés puede bloquear la asociación, dependiente de herregulina, de HER2 con HER3 en ambas células MCF7 y SK-BR-3, como se determina en un experimento de co-inmunoprecipitación tal como el descrito en WO01/00245, sustancialmente en forma más efectiva que el anticuerpo monoclonal 4D5 y, preferiblemente sustancialmente en forma más efectiva que el anticuerpo monoclonal 7F3.

Para identificar anticuerpos HER2 inhibidores de crecimiento se puede cribar para detectar anticuerpos que inhiban el crecimiento de células cancerosas que sobreexpresan HER2. En una incorporación, el anticuerpo

inhibidor de crecimiento de elección es capaz de inhibir el crecimiento de células SK-BR-3 en un cultivo celular en alrededor de 20-100% y, preferiblemente, en alrededor de 50-100% con una concentración de anticuerpo de alrededor de 0.5 a 30 μg/ml. Para identificar tales anticuerpos, puede llevarse a cabo el análisis SK-BR-3 descrito en la Patente de los Estados Unidos No. 5,677,171. De acuerdo con este análisis, células SK-BR-3 se cultivan en una mezcla 1:1 de medio F12 y DMEM suplementado con 10% de suero fetal bobino, glutamina y penicilina en estreptomicina. Las células se esparcen a 20.000 células en una placa de cultivo de células de 35 mm (2 mls/35mm por placa). Por placa se agregan 0.5 a 30 μg/ml del anticuerpo HER2. Después de seis días, se cuenta el número de células, comparado con células no tratadas usando un contador electrónico de células COULTER™. Aquellos anticuerpos que inhiben el crecimiento de las células SK-BR-3 en alrededor de 20-100% o alrededor de 50-100% pueden seleccionarse como compuestos inhibidores de crecimiento. Véase la Patente de los Estados Unidos No. 5,677,171 con respecto a análisis para cribar anticuerpos inhibidores de crecimiento, tales como 4D5 y 3E8.

Con el fin de seleccionar anticuerpos HER2 que inducen apoptosis, se dispone de análisis de unión de anexina usando células BT474. Las células BT474 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 anticuerpo monoclonal. Luego de un período de incubación de tres días, las monocapas se lavan con PBS y se separan por tripsinización. Entonces se centrifugan las células, se resuspenden en amortiguador 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 anticuerpos, que inducen niveles estadísticamente significativos de unión de anexina, en relación con el control, se

seleccionan como anticuerpos que inducen apoptosis. Aparte del análisis de unión de anexina, se dispone de un análisis de tinción de ADN usando células BT474. Con el fin de realizar esta análisis, células BT474 que se han tratado con el anticuerpo de interés, como se describe en los dos párrafos precedentes, se incuban con 9µg/ml HOECHST 33342™ durante 2 horas a 37°C, luego se analizan en un citómetro de flujo EPICS ELITE™ (Coulter Corporation) usando software MODFIT LT™ (Verity Software House). Los anticuerpos que inducen un cambio en el porcentaje de células apoptóticas, que es 2 veces o más (y, preferiblemente, 3 veces o más) que las células no tratadas (células apotóticas hasya el 100%), pueden seleccionarse como anticuerpos pro-apoptóticos usando este análisis. Véase WO98/17797 con respecto a análisis para detectar anticuerpos HER2, que inducen apoptosis, tales como 7C2 y 7F3.

Para cribar anticuerpos, que se unen a un epítopo en HER2 ligado por el anticuerpo de interés, puede realizarse un análisis rutinario de enlazamiento cruzado, tal como el descrito en Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), con el fin de evaluar si el anticuerpo bloquea cruzadamente la unión de un anticuerpo, tal como 2C4 o Pertuzumab, a HER2. Alternativa, o adicionalmente, puede llevarse a cabo un mapeo de epítopos por métodos conocidos en el arte, y/o se puede estudiar la estructura anticuerpo-HER2 (Franklin *et al.* Cancer Cell 5:317-328 (2004)) para ver qué dominio(s) de HER2 se liga/ligan por el anticuerpo.

(ix) Inmunoconjugados

La invención concierne además a inmunoconjugados que comprenden un anticuerpo conjugado a un agente citotóxico tal como un agente quimioterapéutico, toxina (*por ejemplo*, una toxina enzimáticamente activa de origen bacteriano, fúngico, vegetal, o animal, o fragmentos de la misma), o un isótopo radioactivo(*es decir*, un radioconjugado).

Agentes quimioterapéuticos útiles en la generación de tales inmunoconjugados se han descrito más arriba. Conjugados de un anticuerpo y una o más toxinas de molécula pequeña, tales como una

caliqueamicina, una maitansina (Patente de los Estados Unidos No. 5,208,020), un tricoteno, y CC1065 se contemplan igualmente en esta solicitud.

En una incorporación preferida de la invención, el anticuerpo se conjuga con una o más moléculas de maitansina (por ejemplo, alrededor de 1 a alrededor de 10 moléculas de maitansina por molécula de anticuerpo). La maitansina puede, por ejemplo, convertirse en May-SS-Me, el cual puede reducirse a May-SH3 y hacerse reaccionar con anticuerpo modificado (Chari *et al.* Cancer Research 52: 127-131 (1992)), para generar un inmunoconjugado maitansinoide-anticuerpo.

Otro inmunoconjugado de interés comprende un anticuerpo HER2 conjugado con una o más moléculas de caliqueamicina. La familia caliqueamicina de antibióticos es capaz de producir cortes de ADN bicatenario en concentraciones sub-picomolares. Análogos estructurales de caliqueamicina que pueden usarse incluyen, pero no se limitan a, γ_1^I α_2^I, α_3^I, N-acetil- γ_1^I, PSAG y θ_1^I (Hinman *et al., Cancer Research* 53: 3336-3342 (1993) y Lode *et al., Cancer Research* 58: 2925-2928 (1998)). Véase, también, las Patentes de los Estados Unidos No. 5,714,586; 5,712,374; 5,264,586; y 5,773,001 expresamente incorporadas en esta solicitud por referencia.

Toxinas enzimáticamente activas y sus fragmentos, que pueden usarse, incluyen la cadena A de la difteria, fragmentos activos de no unión de toxina de la difteria, la cadena A de exotoxina (de *Pseudomonas aeruginosa)*, la cadena A de ricina, la cadena A de abrina, la cadena A de modeccina, alfa-sarcina, proteínas de *Aleurites fordii*, proteínas de diantina, proteínas de *Phytolaca americana* (PAPI, PAPII, y PAP-S), inhibidor de *momordica charantia*, curcina, crotina, inhibidor de *sapaonaria officinalis*, gelonina, mitogelina, restrictocina, fenomicina, enomicina y los tricotecenos. Véase, por ejemplo, WO 93/21232 publicada el 28 de octubre de 1993.

La presente invención contempla además un inmunoconjugado formado entre un anticuerpo y un compuesto con actividad nucleolítica (por

ejemplo, una ribonucleasa o una ADN endonucleasa tal como una deoxiribonucleasa; DNasa).

Una variedad de isótopos radioactivos se encuentra disponible para la producción de anticuerpos HER2 radioconjugados. Los ejemplos incluyen At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} e isótopos radioactivos de Lu.

Conjugados del anticuerpo y agente citotóxico pueden elaborarse usando una variedad de agentes de acoplamiento de proteínas bifuncionales tales como N-succinimidil-3-(2-piridilditiol) propionato (SPDP), succinimidil-4-(N-maleimidometil)ciclohexano-1-carboxilato, iminotiolano (IT), derivados bifuncionales de imidoésteres (tales como HCl de adipimidato dimetílico), ésteres activos (tales como disuccinimidil suberato), aldehídos (tales como glutareldehído), compuestos bis-azido (tales como bis (p-azidobenzoil)hexanodiamina), derivados de bis-diazonio (tales como bis-(p-diazoniobenzoil)-etilendiamina), diisocianatos (tales como tolien 2,6-diisocianato), y compuestos de flúor bis-activo (tales como 1,5-difluoro-2,4-dinitrobenceno). Por ejemplo, puede prepararse una inmunotoxina de ricina como se describe en Vitetta *et al., Science*, 238: 1098 (1987). Ácido 1-isotiocianatobencil-3-metildietilen triaminapentaacético marcado con carbono 14 (MX-DTPA) es un agente quelante ejemplar para conjugación de radionucleótido al anticuerpo. Véase WO94/11026. El enlazante puede ser un "enlazante segmentable" que facilite la liberación del medicamento citotóxico en la célula. Por ejemplo, pueden usarse un enlazante lábil a ácidos, un enlazante sensible a peptidasa, un enlazante dimetílico o un enlazante que contenga disulfuro (Chari *et al., Cancer Res.* 52:127-131 (1992)).

Alternativamente, puede elaborarse una proteína de fusión que comprenda el anticuerpo HER2 y un agente citotóxico, por ejemplo, mediante técnicas recombinantes o síntesis peptídica.

En todavía otra incorporación, el anticuerpo puede conjugarse a un "receptor" (tal como estreptavidina) para utilización en pre-direccionamiento tumoral, en donde el conjugado anticuerpo-receptor se

administra al paciente, seguido por eliminación del conjugado no ligado de la circulación usando un agente depurador y, en seguida, administración de un "ligando" (*por ejemplo,* avidina) que se conjuga a un agente citotóxico (*por ejemplo*, un radionucleótido).

(x) Otras modificaciones de anticuerpo

Aquí se contemplan otras modificaciones de anticuerpos. Por ejemplo, el anticuerpo puede enlazarse a uno de una variedad de polímeros no proteináceos, *por ejemplo*, polietilen glicol, polipropilen glicol, polioxialquilenos, o copolímeros de polietilen glicol y polipropilen glicol. En igual forma, el anticuerpo puede atraparse en microcápsulas preparadas, por ejemplo, por técnicas de aglomeración o por polimerización interfacial (por ejemplo, hidroximetilcelulosa o microcápsulas de gelatina y microcápsulas de poli-(metilmetacrilato), respectivamente), en sistemas coloidales de administración de medicamentos (por ejemplo, liposomas, microesferas de albúmina, microemulsiones, nanopartículas y nanocápsulas), o en macroemulsiones. Tales técnicas y otras formulaciones apropiadas se divulgan en *Remington's Pharmaceutical Sciences*, 16th edition, Oslo, A., Ed., (1980).

Puede ser deseable modificar el anticuerpo de la invención con respecto a la función efectora, *por ejemplo*, con el fin de intensificar la citotoxicidad celular dependiente de antígeno (ADCC) y/o la citotoxicidad dependiente de complemento (CDC) del anticuerpo. Esto puede lograrse introduciendo una o más sustituciones de aminoácidos en una región Fc del anticuerpo. Alternativa, o adicionalmente, puede(n) introducirse en la región Fc un residuo(s) de cisteína, permitiendo así la formación de enlaces disulfuro intracadena en esta región. El anticuerpo homodimérico así generado puede tener una capacidad de internalización mejorada y/o creciente eliminación celular mediada por complemento y citotoxicidad celular dependiente del anticuerpo (ADCC). Véase Caron *et al., J. Exp. Med.* 176:1191-1195 (1992) y Shopes, *J. Immunol.* 148: 2918-2922 (1992). Anticuerpos homodiméricos con actividad antineoplásica mejorada pueden prepararse también usando entrecruzadores heterobifuncionales como se

describe en Wolff *et al., Cancer Research* 53: 2560-2565 (1993). Alternativamente, puede diseñarse un anticuerpo que tenga regiones Fc duales y pueda, por ello, tener lisis de complemento mejorada y posibilidades ADCC. Véase Stevenson *et al., Anti-Cancer Drug Design* 3:219-230 (1989).

WO00/42072 (Presta, L.) describe anticuerpos con función ADCC mejorada en la presencia de células efectoras humanas, donde los anticuerpos comprenden sustituciones de aminoácidos en su región Fc. Preferiblemente, el anticuerpo con ADCC mejorada comprende sustituciones en las posiciones 298, 333, y/o 334 de la región Fc. Preferiblemente la región Fc alterada es una región Fc de IgG1 humana, que comprende o consta de sustituciones en una, dos o tres de estas posiciones.

Anticuerpos con unión C1q alterada y/o citotoxicidad dependiente de complemento (CDC) se describen en WO99/51642, Patente de los Estados Unidos No. 6,194,551Bl, Patente de los Estados Unidos No. 6,242,195Bl, Patente de los Estados Unidos No. 6,528,624Bl y Patente de los Estados Unidos No. 6,538,124 (Idusogie *et al.*). Los anticuerpos comprenden una sustitución de aminoácido en una o más de las posiciones de aminoácidos 270, 322, 326, 327, 329, 313, 333 y/o 334 de la región Fc de los mismos.

Para incrementar la semivida sérica del anticuerpo, se puede incorporar un epítopo de unión a receptor de salvamento al anticuerpo (especialmente un fragmento de anticuerpo), como se describe en la Patente de los Estados Unidos 5,739,277, 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. Anticuerpos con sustituciones en una región Fc de los mismos y semividas incrementadas se describen también en WO00/42072 (Presta, L.).

También se contemplan anticuerpos genéticamente manipulados con tres o más (preferiblemente, cuatro) sitios de unión funcional a antígeno (Solicitud de los Estados Unidos No. US2002/0004587 A1, Miller *et al.*).

Los anticuerpos HER2 divulgados en esta solicitud pueden formularse además como liposomas. Los liposomas que contienen el anticuerpo se preparan por métodos conocidos en el arte, tales como los descritos en Epstein *et al., Proc. Natl. Acad. Sci. USA,* 82:3688 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77: 4030 (1980); Patente de los Estados Unidos No. 4,485,045 y 4,544,545; y WO 1997/38731 publicada el 23 de octubre de 1997. Liposomas con tiempo de circulación mejorado se divulgan en la Patente de los Estados Unidos No. 5,013,556.

Liposomas particularmente útiles pueden generarse por el método de evaporación de fase reversa con una composición lipídica que comprenda fosfatidilcolina, colesterol, y fosfatidiletanolamina derivada de PEG (PEG-PE). Los liposomas se extruyen a través de filtros de tamaño de poro definido para proporcionar liposomas con el diámetro deseado. Fragmentos Fab' del anticuerpo de la presente invención pueden conjugarse a los liposomas como se describe en Martin *et al., J. Biol. Chem.*, 257:286-288 (1982) mediante una reacción de intercambio de disulfuro. Un agente quimioterapéutico se encuentra contenido opcionalmente en el liposoma. Véase Gabizon *et al., J. National Cancer Inst.* 81(19):1484(1989).

(ix) Anticuerpos ejemplares

Anticuerpos ejemplares que pueden formularse de acuerdo con la presente invención incluyen, pero no se limitan a los siguientes: anticuerpos anti-ErbB, incluidos anticuerpos anti-HER2, tales como los descritos con más detalle en esta solicitud; anticuerpos que se unen a un marcador de superficie de linfocito B, tal como CD19, CD20 (por ejemplo Rituximab (RITUXAN®) y 2H7 humanizado), CD22, CD40 ó BR3; anticuerpos que se unen a IgE, incluidos Omalizumab (XOLAIR®) disponible comercialmente de Genentech, E26 (Figs. 17A-B en esta solicitud), HAE1 (Figs. 17A-B en esta solicitud), anticuerpo IgE con una sustitución de aminoácidos en la posición 265 de una región Fc del mismo (US 2004/0191244 A1), Hu-901 (Figs. 17A-B en esta solicitud), un anticuerpo IgE como en WO2004/070011, o un anticuerpo (incluidos fragmentos de anticuerpo y anticuerpos de longitud total) que comprenden los dominios

variables de cualquiera de estos anticuerpos IgE. Véase, asímismo, Presta *et al.*, *J. Immunol.* 151:2623-2632 (1993); Publicación Internacional No. WO 95/19181; Patente de los Estados Unidos No. 5,714,338, expedida el 3 de febrero de 1998; Patente de los Estados Unidos No. 5,091,313, expedida el 25 de febrero de 1992; WO 93/04173 publicada el 4 de marzo de 1993; WO 99/01556 publicada el 14 de enero de 1999; y Patente de los Estados Unidos No. 5,714,338; anticuerpos que se unen al factor de crecimiento endotelial vascular (VEGF) o un receptor del mismo, incluidos Bevacizumab (AVASTIN™), disponible comercialmente de Genentech, y Ranibizumab (LUCENTIS™); anticuerpos anti-IL-8 (St. John *et al.*, *Chest*, 103:932 (1993), y Publicación Internacional No. WO 95/23865); anticuerpos anti-PSCA (WO01/40309); anticuerpos anti-CD40, incluido S2C6 y variantes humanizadas de los mismos (WOOO/75348); anticuerpos anti-CD11a, incluidos efalizumab (RAPTIVA®) (Patente de los Estados Unidos No. 5,622,700, WO 98/23761, Steppe *et al.*, *Transplant Intl.* 4:3-7 (1991), y Hourmant *et al.*, *Transplantation* 58:377-380 (1994)); anticuerpos anti-CD18 (Patente de los Estados Unidos No. 5,622,700, expedida el 22 de abril de 1997, o como en WO 97/26912, publicada el 31 de julio de 1997); anticuerpo de receptor anti-Apo-2 (WO 98/51793 publicada el 19 de noviembre de 1998); anticuerpos anti-TNF-α cA2 (REMICADE®), CDP571 y MAK-195 (véase, Patente de los Estados Unidos No. 5,672,347 expedida el 30 de septiembre de 1997, Lorenz *et al.*, *J. Immunol.* 156(4):1646-1653 (1996), y Dhainaut *et al.*, *Crit. Care Med.* 23(9):1461-1469 (1995)); Factor anti-Tisular (TF) (Patente Europea No. 0420 937 Bl otorgada el 9 de noviembre de 1994); integrina α4β7 anti-humana (WO 98/06248 publicada el 19 de febrero de 1998); anticuerpos anti-EGFR, incluido el anticuerpo 225 quimérico o humanizado como en WO 96/40210 publicada el 19 de diciembre de 1996; anticuerpos anti-CD3, tales como OKT3 (Patente de los Estados Unidos No. 4,515,893 expedida el 7 de mayo de 1985); anticuerpos anti-CD25 o anti-tac tales como CHI-621 (SIMULECT®) y (ZENAPAX®) (véase Patente de los Estados Unidos No. 5,693,762 expedida el 2 de diciembre de 1997); anticuerpos anti-CD4 tales

como el anticuerpo cM-7412 (Choy *et al.*, *Arthritis Rheum.* 39(l):52-56 (1996)); anticuerpos anti-CD52 tales como CAMPATH-1H (Riechmann *et al.*, *Nature* 332:323-337 (1988); anticuerpos de receptor anti-Fc tales como el anticuerpo M22 dirigido contra FcγRI como en Graziano *et al.*, *J. Immunol.* 155(10):4996-5002 (1995); anticuerpos de antígeno anti-carcinoembrionario (CEA) tales como hMN-14 (Sharkey *et al.*, *Cancer Res.* 55 (23 suplemento): 5935s-5945s (1995); anticuerpos dirigidos contra células epiteliales de mama, incluidos huBrE-3, hu-Mc 3 y CHL6 (Ceriani *et al.*, *Cancer Res.* 55(23): 5852s-5856s (1995); y Richman *et al.*, *Cancer Res.* 55(23 suplemento): 5916s-592Os (1995)); anticuerpos que se unen a células de carcinoma de colon tales como C242 (Litton *et al.*, *Eur J. Immunol.* 26 (l): 1-9 (1996)); anticuerpos anti-CD38, por ejemplo AT 13/5 (Ellis *et al.*, *J. Immunol.* 155(2):925-937 (1995)); anticuerpos anti-CD33 tales como Hu M195 (Jurcic *et al.*, *Cancer Res.* 55(23 suplemento): 5908s-5910s (1995) y CMA-676 ó CDP771; anticuerpos anti-CD22 tales como LL2 o LymphoCide (Juweid *et al.*, *Cancer Res.* 55 (23 suplemento):5899s-5907s (1995); anticuerpos anti-EpCAM tales como 17-1A (PANOREX®); anticuerpos anti-GpIIb/IIIa tales como abciximab o c7E3 Fab (REOPRO®); anticuerpos anti-RSV tales como MEDI-493 (SYNAGIS®); anticuerpos anti-CMV tales como PROTOVTR®; anticuerpos anti-HIV tales como PRO542; anticuerpos anti-hepatitis tales como el anticuerpo anti-Hep B OSTAVIR®; anticuerpo anti-CA 125 OvaRex; anticuerpo BEC2 de epítopo anti-idiotípico GD3; anticuerpo anti-αvβ3 VITAXIN®; anticuerpo de carcinoma de células renales anti-humano tal como ch-G250; ING-1; anticuerpo anti-humano 17-1A (3622W94); anticuerpo de tumor colorrectal anti-humano (A33); anticuerpo de melanoma anti-humano R24 dirigido contra el ganglιósido GD3; anticuerpos de carcinoma escamoso anti-humano (SF-25); y anticuerpos de antígeno de leucocitos anti-humano (HLA) tales como Smart ID10 y el anticuerpo anti-HLA DR Oncolym (Lym-1).

(xi) Composiciones de variantes de anticuerpo

La presente invención, en por lo menos un aspecto, concierne a formulaciones que comprenden una composición que contiene una mezcla de un anticuerpo de especie principal y una o más variantes del mismo. Donde el anticuerpo de especie principal une HER2, preferiblemente el anticuerpo HER2 (cualquiera o ambos de los anticuerpos HER2 de especie principal y su variante de anticuerpo) es uno que se une al Dominio II de HER2, inhibe la dimerización HER más efectivamente que Trastuzumab, y/o se une a un sitio heterodimérico de unión de HER2. La incorporación preferida en esta solicitud del anticuerpo de especie principal es uno que comprende las secuencias de aminoácidos pesadas variables y ligeras variables en SEQ ID Nos. 3 y 4 y, muy preferiblemente, que comprende la secuencia de aminoácidos de cadena ligera seleccionada de SEQ ID No. 15 y 23, y una secuencia de aminoácidos de cadena pesada seleccionada de SEQ ID No. 16 y 24.

En una incorporación, la composición de anticuerpo HER2 formulada comprende una mezcla del anticuerpo HER2 de especie principal y una variante de secuencia de aminoácidos del mismo que contiene una extensión líder amino-terminal. Preferiblemente, la extensión líder amino-terminal es una cadena ligera de la variante de anticuerpo (por ejemplo en una o dos cadenas ligeras de la variante de anticuerpo). El anticuerpo HER2 de especie principal o la variante de anticuerpo puede ser un anticuerpo de cadena total o fragmento de anticuerpo (por ejemplo, fragmentos Fab de $F(ab')_2$), pero preferiblemente ambos son anticuerpos de longitud total. La variante de anticuerpo en esta solicitud puede comprender una extensión líder amino-terminal en una cualquiera o más de sus cadenas ligeras o pesadas. Preferiblemente, la extensión líder amino-terminal es una o dos cadenas ligeras del anticuerpo. Preferiblemente, la extensión líder amino-terminal comprende o consta de VHS. La presencia de la extensión líder amino-terminal en la composición puede detectarse por varias técnicas analíticas incluidas, pero no limitadas a, análisis de secuencia N-terminal, análisis de heterogeneidad de carga (por ejemplo,

cromatografía de intercambio catiónico o electroforesis de zona capilar), espectrometría de masas, etc. La cantidad de la variante de anticuerpo en la composición varía generalmente desde una cantidad, que constituye el límite de detección de cualquier análisis (preferiblemente análisis de secuencia N-terminal) utilizado para detectar la variante, hasta una cantidad menor que la cantidad de anticuerpo de especie principal. Generalmente, alrededor de 20% o menos (por ejemplo, desde alrededor de 1% a alrededor de 15%, por ejemplo desde 5% a alrededor de 15%) de las moléculas del anticuerpo en la composición comprenden una extensión líder amino-terminal. Tales cantidades porcentuales se determinan preferiblemente usando análisis cuantitativo de secuencia N-terminal o análisis de intercambio catiónico (preferiblemente usando una columna de intercambio catiónico débil, de alta resolución, tal como un columna de intercambio catiónico PROPAC WCX- 10™). Aparte de la variante de extensión líder amino-terminal, se contemplan alteraciones adicionales de secuencia de aminoácidos del anticuerpo de especie principal y/o variante, incluidos pero no limitados a, un anticuerpo que comprende un residuo lisina C-terminal en una o ambas de sus cadenas pesadas, una variante de anticuerpo desaminado, etc.

Más aún, el anticuerpo de especie principal o variante puede comprender además variaciones de glucosilación, ejemplos no limitantes de las cuales incluyen el anticuerpo HER2 que comprende una estructura de oligosacárido G1 o G2 unida a su región Fc, anticuerpo HER2 que comprende una fracción carbohidrato unida a su cadena ligera (por ejemplo, una o dos fracciones carbohidrato unidas a una o dos cadenas ligeras del anticuerpo), anticuerpo HER2 que comprende una cadena pesada no glucosilada.

III. Preparación de la Formulación

La presente invención provee, en un primer aspecto, una formulación farmacéutica estable que comprende un anticuerpo monoclonal, preferiblemente un anticuerpo IgG1 humano o humanizado de longitud

total, en amortiguador acetato de histidina, pH 5.5 a 6.5, preferiblemente pH 5.8 a 6.2. No obstante, el anticuerpo en la formulación puede ser un fragmento de anticuerpo que comprende una región de unión a antígeno, tal como un fragmento Fab o $F(ab')_2$.

En otra incorporación, la invención concierne a una formulación farmacéutica que comprende, o consta esencialmente de, un anticuerpo IgG1 de longitud total, susceptible a desamidación o agregación en una cantidad desde alrededor de 10mg/mL a alrededor de 250mg/mL; amortiguador acetato de histidina, pH 5.5 a 6.5; sacárido seleccionado del grupo conformado por trehalosa y sacarosa, en una cantidad desde alrededor de 60mM a alrededor de 250mM; y polisorbato 20 en una cantidad desde alrededor de 0.01% a alrededor de 0.1%.

En todavía otra incorporación, la invención provee una formulación farmacéutica que comprende un anticuerpo que se une al dominio II de HER2 en un amortiguador histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido y un surfactante. Por ejemplo, la formulación puede comprender Pertuzumab en una cantidad desde alrededor de 20mg/mL a alrededor de 40mg/mL, amortiguador acetato de histidina, sacarosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5

En otro aspecto, la invención provee una formulación farmacéutica que comprende un anticuerpo DR5 en un amortiguador histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido y un surfactante. Por ejemplo, tal formulación puede comprender Apomab en una cantidad desde alrededor de 10mg/mL a alrededor de 30mg/mL, amortiguador acetato de histidina, trehalosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5.

La formulación es especialmente útil para anticuerpos que son susceptibles a desamidación y/o agregación y/o fragmentación, porque el amortiguador retarda la desamidación y/o agregación y/o fragmentación del anticuerpo formulado en ella. Adicionalmente, a diferencia de otros amortiguadores de histidina preparados usando HCl, el amortiguador

acetato de histidina carece del ión cloruro, lo que se descubrió que es benéfico en este caso, en el sentido que este amortiguador, cuando se combinó con sacárido, tuvo el mismo efecto protector sobre un anticuerpo que el polisorbato 20, y fue estable y compatible con el almacenamiento en recipientes de acero inoxidable. En consecuencia, además de la formulación *per se*, que comprende el anticuerpo susceptible a desamidación, agregación y/o fragmentación, la invención provee un método para reducir la desamidación, agregación y/o fragmentación de un anticuerpo monoclonal terapéutico (por ejemplo, relativas a una composición con un pH diferente o en un amortiguador diferente), que comprende formular el anticuerpo en un amortiguador de acetato de histidina, pH 5.5 a 6.5. En esta incorporación, se puede determinar la desamidación, agregación y/o fragmentación antes y después de formular el anticuerpo, demostrando el anticuerpo formulado desamidación, agregación y/o fragmentación aceptables en la formulación y luego de su almacenamiento.

El anticuerpo en la formulación puede unir un antígeno, incluidos pero no limitados a: HER2, CD20, IgE, DR5, BR3 y VEGF.

Donde el anticuerpo formulado une HER2, éste es preferiblemente uno que se une al Dominio de HER2, inhibe la dimerización HER más efectivamente que Trastuzumab, y/o se une a un sitio heterodimérico de unión de HER2. La incorporación, preferida en esta solicitud, de un anticuerpo HER2 formulado es uno que comprende las secuencias de aminoácidos pesadas variables y ligeras variables en SEQ ID Nos. 3 y 4 y, muy preferiblemente, que comprende las secuencias de aminoácidos de cadena pesada y cadena ligera en SEQ ID Nos. 15 y 16 (Pertuzumab).

Ejemplos de anticuerpos CD20 que pueden formularse en esta solicitud incluyen: "C2B8" que actualmente se denomina "Rituximab" ("RITUXAN®") disponible comercialmente de Genentech (véase también Patente de los Estados Unidos No. 5,736,137); el anticuerpo murino 2B8 marcado con itrio-[90], designado "Y2B8" o "Ibritumotnab Tiuxetan"

ZEVALIN obtenible comercialmente de Biogen-Idec (véase igualmente la Patente de los Estados Unidos No. 5,736,137, expresamente incorporada aquí por referencia); IgG2a "B1" murino, también llamado "Tositumomab," opcionalmente marcado con ^{131}I para generar el anticuerpo "^{131}I-B1" (tositumomab yodo I^{131}, BEXXAR™) (Patente de los Estados Unidos No. 5,595,721, expresamente incorporada aquí por referencia); anticuerpo monoclonal murino"1F5" (*Press et al., Blood* 69(2):584-591 (1987)) y sus variantes, incluidos IF5 ***"framework patched"*** o humanizado (WO 2003/002607, Leung, S.) ATCC Depósito HB-96450); anticuerpo 2H7 murino y 2H7 quimérico (Clark *et al., PNAS* 82: 1766-1770 (1985); Patente de los Estados Unidos No. 5,500,362, expresamente incorporada aquí por referencia); 2H7 humanizado; huMax-CD20 (WO 04/035607, Genmab, Denmark); AME-133 (Applied Molecular Evolution); anticuerpo A20 o sus variantes tales como anticuerpo A20 quimérico o humanizado (cA20, hA20, respectivamente) (US 2003/0219433, Immunomedics); y anticuerpos monoclonales L27, G28-2, 93-1B3, B-Cl o NU-B2 disponibles de the International Leukocyte Typing Workshop (Valentine *et al.*, In: Leukocyte Typing III (McMichael, Ed., p. 440, Oxford University Press (1987)).

En la incorporación preferida de un anticuerpo CD20 formulado, el anticuerpo CD20 es un anticuerpo 2H7 humanizado. Anticuerpos 2H7 humanizados preferidos en esta solicitud son 2H7v16 y 2H7v511. El 2H7v16 humanizado puede ser un anticuerpo intacto o fragmento de anticuerpo que comprende las secuencias pesadas variables y ligeras variables en Figs. 18A-B (SEQ ID Nos. 26 y 29). Donde el anticuerpo 2H7v16 humanizado es un anticuerpo de longitud total, preferiblemente comprende las secuencias de aminoácidos de cadenas pesadas y ligeras con SEQ ID Nos. 63 y 65.

Donde el anticuerpo une VEGF, preferiblemente comprende las secuencias de dominio variable como se ilustra en Fig. 19. El anticuerpo anti-VEGF muy preferido es el anticuerpo humanizado IgG1 de cadena total, Bevacizumab (AVASTIN™), comercialmente disponible de Genentech.

Donde el anticuerpo formulado une IgE, se selecciona preferiblemente del grupo conformado por: E25, Omalizumab (XOLAIR®) comercialmente disponible de Genentech (véanse además las Figs. 17A-B), E26 (Figs. 17A-B en esta solicitud), HAEl (Figs. 17A-B en esta solicitud), anticuerpo IgE con una sustitución de aminoácido en la posición 265 de su región Fc (US 2004/0191244 Al), Hu-901 (Figs. 17A-B en esta solicitud), un anticuerpo IgE como en WO200M7070011, o un anticuerpo (incluidos fragmentos de anticuerpo y anticuerpos de longitud total) que comprende los dominios variables de cualquiera de esos anticuerpos IgE.

Donde el anticuerpo se une a un receptor en la superfamilia de factor de necrosis tumoral (TNF) o a un receptor de muerte, se une preferiblemente a DR5, y preferiblemente es un anticuerpo agonista. Publicaciones en esta área incluyen Sheridan *et al., Science,* 277:818-821 (1997), Pan *et al., Science,* 277:815-818 (1997), WO98/51793 publicada el 19 de noviembre de 1998; WO98/41629 publicada el 24 de septiembre de1998; Screaton *et al., Curr. Biol.,* 7:693-696 (1997); Walczak *et al., EMBO J.,* 16:5386-5387 (1997); Wu *et al., Nature Genetics,* 17:141-143 (1997); WO98/35986 publicada el 20 de agosto de 1998; EP870,827 publicada el 14 de octubre de 1998; WO98/46643 publicada el 22 de octubre de 1998; WO99/02653 publicada el 21 de enero de 1999; WO99/09165 publicada el 25 de febrero de 1999; WO99/11791 publicada el 11 de marzo de 1999; US 2002/0072091 publicada el 13 de agosto de 2002; US 2002/0098550 publicada el 7 de diciembre de 2001; US 6,313,269 expedida 6 de diciembre de 2001; US 2001/0010924 publicada el 2 de agosto de 2001; US 2003/01255540 publicada el 3 de julio de 2003; US 2002/0160446 publicada el 31 de octubre de 2002, US 2002/0048785 publicada el 25 de abril de 2002; US 6,342,369 expedida en febrero de 2002; US 6,569,642 expedida 27 de mayo de 2003, US 6,072,047 expedida 6 de junio de 2000, US 6,642,358 expedida el 4 de noviembre de 2003; US 6,743,625 expedida el 1 junio de 2004. El anticuerpo DR5 muy preferido es Apomab.

Cada una de las formulaciones ya anotadas comprende un amortiguador, preferiblemente un amortiguador de histidina y, muy preferiblemente, un amortiguador de acetato de histidina con un pH de 5.5 a 6.5, preferiblemente 5.8 a 6.2, por ejemplo aproximadamente 6.0. La concentración del amortiguador es dictada, por lo menos en parte, por el pH deseado. Concentraciones ejemplares para el amortiguador están en el rango desde alrededor de 1mM a alrededor de 200mM, preferiblemente desde alrededor de 10mM a alrededor de 40mM, muy preferiblemente alrededor de 20mM.

La concentración de anticuerpo en la formulación está preferiblemente en el rango desde alrededor de 10mg/mL a alrededor de 250mg/mL. La concentración de anticuerpo puede determinarse basándose en el uso previsto y el modo de administración de la formulación. Por ejemplo, donde la formulación es para administración i.v. (por ejemplo, un anticuerpo HER2), la concentración de anticuerpo en la formulación es preferiblemente desde alrededor de 20mg/mL a alrededor de 40mg/mL. En la formulación ejemplificada de Pertuzumab prevista para administración intravenosa (i.v.), la concentración de anticuerpo fue desde alrededor de 20mg/mL a alrededor de 40mg/mL, muy preferiblemente alrededor de 30mg/mL.

Donde el anticuerpo es para administración SQ o IM (por ejemplo, para un anticuerpo anti-IgE) pueden desearse concentraciones más elevadas del anticuerpo. Tales concentraciones de anticuerpo sustancialmente elevadas pueden ser desde alrededor de 50mg/mL a alrededor de 250mg/mL, o desde alrededor de 80mg/mL a alrededor de 250mg/mL, o desde alrededor de 100mg/mL a alrededor de 200mg/mL.

Donde la formulación comprende un anticuerpo DR5, tal como Apomab, concentraciones ejemplares de anticuerpo son desde alrededor de 10mg/mL a alrededor de 30mg/mL, por ejemplo alrededor de 20mg/mL de anticuerpo DR5; siendo útil tal formulación para administración intravenosa.

La formulación para administración es preferiblemente una formulación acuosa (no liofilizada) y no se ha sometido a liofilización previa. Aunque la formulación puede liofilizarse, preferiblemente no se liofiliza. No obstante, el congelamiento de la formulación acuosa, sin secamiento simultáneo que ocurre durante el secamiento por congelamiento, se contempla específicamente en esta solicitud, facilitando su almacenamiento a más largo plazo, por ejemplo en un recipiente de acero inoxidable.

Además, la formulación comprende preferiblemente un sacárido, muy preferiblemente un disacárido, tal como trehalosa o sacarosa. El sacárido se incluye generalmente en una cantidad que reduce la formación de agregados solubles, tal como la que ocurre luego de congelamiento/descongelamiento. Concentraciones ejemplares de sacárido están en el rango desde alrededor de 10mM a alrededor de 1M, por ejemplo desde alrededor de 60mM a alrededor de 250mM y, muy preferiblemente, alrededor de 120mM para una formulación de anticuerpo HER2 y, alrededor de 240mM para una formulación de anticuerpo DR5.

Aunque se ha descubierto en esta solicitud que una formulación que comprende amortiguador de acetato de histidina y sacárido era estable, la formulación comprende además opcionalmente un surfactante, tal como polisorbato, muy preferiblemente polisorbato 20. El surfactante se incluye generalmente en una cantidad que reduzca la formación de agregados insolubles (tal como la que ocurre luego de agitar). La concentración de surfactante es preferiblemente desde alrededor de 0.0001% a alrededor de 1.0%, muy preferiblemente desde alrededor de 0.01% a alrededor de 0.1%, por ejemplo alrededor de 0.02%.

Opcionalmente, la formulación no contiene una cantidad tonicificante de una sal, tal como cloruro de sodio.

La formulación es generalmente estéril, y esto puede lograrse, de acuerdo con los procedimientos conocidos por la persona experta, para generar formulaciones farmacéuticas estériles apropiadas para

administración a sujetos humanos, incluida filtración a través de membranas de filtración estéril, antes o luego de la preparación de la formulación.

Más aún, deseablemente la formulación es una que se ha demostrado que es estable luego del almacenamiento. Se dispone de varios análisis de estabilidad al alcance del profesional experto para confirmar la estabilidad de la formulación. Por ejemplo, la formulación puede ser una que se haya descubierto que es estable luego del almacenamiento: a alrededor de 40°C durante por lo menos 4 semanas; a alrededor de 5°C o alrededor de 15°C durante por lo menos 3 meses o por lo menos 1 año; y/o alrededor de -20°C durante por lo menos 3 meses. La estabilidad puede probarse evaluando la estabilidad física, la estabilidad química, y/o la actividad biológica del anticuerpo en la formulación alrededor del tiempo de formulación, así como luego del almacenamiento a las temperaturas anotadas. La estabilidad física y/o química puede evaluarse cualitativa y/o cuantitativamente en una variedad de diferentes modos, incluida la evaluación de formación de agregados (por ejemplo usando cromatografía de exclusión de tamaño, midiendo la turbiedad, y/o por inspección visual); evaluando la heterogeneidad de carga usando cromatografía de intercambio catiónico o electroforesis de zona capilar; análisis de secuencia amino-terminal o carboxi-terminal; análisis espectrométrico de masas; análisis SDS-PAGE para comparar anticuerpo intacto y reducido; análisis por mapeo peptídico (por ejemplo tríptico o LYS-C); evaluar la actividad biológica o función de unión a antígeno del anticuerpo; etc. La inestabilidad puede resultar en agregación, desamidación (por ejemplo, desamidación Asn), oxidación (por ejemplo, oxidación Met), isomerización (por ejemplo, isomerización Asp), corte/hidrólisis/fragmentación (por ejemplo, fragmentación de la región de unión flexible), formación de succinimida, cisteína(s) no apareada, extensión N-terminal, procesamiento C-terminal, diferencias de glucosilación, etc. La actividad biológica o la función de unión a antígeno pueden evaluarse usando varias técnicas al alcance del profesional experto.

Como ya se anotó, el congelamiento de la formulación se contempla específicamente en esta solicitud. En consecuencia, la formulación puede

probarse con respecto a estabilidad luego del congelamiento y descongelamiento.

Por lo tanto, la invención provee asimismo un método de elaborar una formulación farmacéutica, que comprende preparar la formulación como se describe en esta solicitud, y evaluar la estabilidad física, la estabilidad química, o la actividad biológica del anticuerpo monoclonal en la formulación.

En la incorporación preferida, la formulación se provee dentro de un frasquito con un tapón perforable por una jeringa, preferiblemente en forma acuosa. Deseablemente, el frasquito se almacena a alrededor de 2-8°C hasta cuando se administra a un sujeto necesitado de ello. El frasquito puede ser por ejemplo un vial de 20cc (por ejemplo para una dosis de 420mg) o un frasquito de 50cc (por ejemplo para una dosis de 1.050mg). Para un anticuerpo DR5, tal como Apomab, la formulación puede proveerse en un frasquito de vidrio de 5cc (por ejemplo, 5.5ml de capacidad).

En otra incorporación, la formulación se provee dentro de un recipiente de acero inoxidable. Opcionalmente, la formulación en el recipiente de acero inoxidable se congela y no se seca por congelamiento.

Uno o más vehículos, excipientes o estabilizantes farmacéuticamente aceptables, tales como los descritos en Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) pueden incluirse en la formulación, siempre que no afecten adversamente las características deseadas de la formulación. Vehículos, excipientes o estabilizantes aceptables son no tóxicos para los recipiendarios en las posologías y concentraciones empleadas e incluyen: agentes amortiguadores adicionales; co-disolventes; antioxidantes incluidos ácido ascórbico y metionina; agentes quelantes tales como EDTA; complejos de metal (por ejemplo, complejos Zn-proteína); polímeros biodegradables tales como poliésteres; preservantes; y/o iones contrarios formadores de sales, tales como sodio.

IV. Tratamiento con la Formulación de Anticuerpo

En una incorporación, la invención provee un método de tratar una enfermedad o trastorno en un sujeto, método que comprende administrar la formulación descrita en esta solicitud a un sujeto en una cantidad efectiva para tratar la enfermedad o trastorno.

Donde el anticuerpo en la formulación se une a HER2, se usa preferiblemente para tratar cáncer. El cáncer comprenderá generalmente células que expresan HER2, de modo que el anticuerpo HER2 en esta solicitud está en capacidad de unirse a las células cancerosas. En consecuencia, la invención en esta incorporación concierne a un método para tratar cáncer que expresa HER2 en un sujeto, método que comprende administrar la formulación farmacéutica del anticuerpo HER2 al sujeto en una cantidad efectiva para tratar cáncer. Varias neoplasias malignas que pueden tratarse con la composición se enumeran en la sección Definiciones de más arriba.

Se contempla asimismo que la formulación de anticuerpo HER2 pueda usarse para tratar varias enfermedades no malignas o trastornos, tales como una enfermedad autoinmunitaria (por ejemplo, psoriasis); endometriosis; escleroderma; restenosis; pólipos tales como pólipos del colon, pólipos nasales o pólipos gastrointestinales; fibroadenoma; enfermedad respiratoria (véase definición más arriba); colecistitis; neurofibromatosis; enfermedad de riñón poliquístico; enfermedades inflamatorias; trastornos de la piel incluidas psoriasis y dermatitis; vasculopatía (véase definición más arriba); afecciones que involucran proliferación anormal de las células epiteliales vasculares; úlceras gastrointestinales; enfermedad de Ménétrier, adenomas secretores o síndrome de pérdida de proteína; trastornos renales; trastornos angiogénicos; enfermedad ocular tal como degeneración macular relacionada con la edad, síndrome de presunta histoplasmosis ocular, neovascularización retiniana de retinopatía diabética proliferativa, vascularización retiniana, retinopatía diabética, o degeneración macular relacionada con la edad; patologías asociadas con los huesos tales como

osteoartritis, raquitismo y osteoporosis; daño luego de un episodio isquémico cerebral; enfermedades fibróticas o de edemia tales como cirrosis hepática, fibrosis pulmonar, sarcoidosis, tiroiditis, síndrome sistémico por hiperviscosidad, enfermedad de Osler Weber-Rendu, enfermedad pulmonar oclusiva crónica, o edema a raíz de quemaduras, trauma, radiación, apoplejía, hipoxia o isquemia; reacción de hipersensibilidad de la piel; retinopatía diabética y nefropatía diabética; síndrome de Guillain-Barré; enfermedad de injerto contra el anfitrión o rechazo de transplante; enfermedad de Paget; inflamación de los huesos o las articulaciones (***"bone or joint inflammation"***); fotoenvejecimiento (por ejemplo, causado por radiación UV de la piel humana); hipertrofia prostática benigna; ciertas infecciones microbianas incluidos patógenos microbianos seleccionados de adenovirus, hantavirus, Borrelia burgdorferi, Yersinia spp. y Bordetella pertussis; trombos causados por agregación plaquetaria; afecciones reproductivas tales como endometriosis, síndrome de hiperestimulación ovárica, preeclampsia, metrorragia funcional, o menometrorragia; sinovitis; ateroma; nefropatías agudas y crónicas (incluidas glomerulonefritis proliferativa y nefropatía inducida por diabetes); eccema; formación de cicatrices hipertróficas; choque endotóxico e infección fúngica; poliposis por adenomatosis familiar; enfermedades neurodegenerativas (por ejemplo, enfermedad de Alzheimer, demencia relacionada con SIDA, enfermedad de Parkinson, esclerosis lateral amiotrófica, retinitis pigmentosa, atrofia muscular espinosa y degeneración cerebelar); síndromes mielodisplásicos; anemia aplásica; lesión isquémica; fibrosis del pulmón, riñón o hígado; enfermedad de hipersensibilidad mediada por linfocitos T; estenosis pilórica hipertrófica infantil; síndrome obstructivo urinario; artritis psoriática; y tiroiditis de Hashimoto. Indicaciones no malignas preferidas para terapia en esta solicitud incluyen psoriasis, endometriosis, escleroderma, vasculopatía (por ejemplo, restenosis, arteriosclerosis, arteriopatía coronaria, o hipertensión), pólipos del colon, fibroadenoma o enfermedad respiratoria (por ejemplo, asma, bronquitis crónica, bronquiectasia o fibrosis quística).

Donde el anticuerpo en la formulación se une a un marcador de superficie de linfocito B tal como CD20 ó B R3, la formulación puede usarse para tratar una neoplasia maligna de linfocitos B, tal como NHL o CLL, una enfermedad autoinmunitaria, rechazo de injerto, o para bloquear una respuesta inmunitaria a un antígeno extraño, tal como un anticuerpo, una toxina, un vector vírico de terapia génica, un injerto, un agente infeccioso, o un aloantígeno (véase WO 01/03734, Grillo-Lopez *et al.*).

Donde el anticuerpo en la formulación es un anticuerpo IgE, puede usarse para tratar un trastorno mediado por IgE (USSN 2004/0197324 Al, Liu and Shire), tal como asma alérgico, rinitis alérgica, dermatitis atópica, gastroenteropatía alérgica, hipersensibilidad, eccema, urticaria, aspergilosos broncopulmonar alérgica, enfermedad parasitaria, síndrome de hiper-IgE, ataxia-telangiectasia, síndrome de Wiskott-Aldrich, alinfoplasia tímica, mieloma IgE, y reacción injerto contra el anfitrión.

Anticuerpos que se unen a un receptor en la superfamilia TNF (por ejemplo, que se unen a DR5), o que se unen a VEGF (o a un receptor del mismo), pueden usarse para tratar cáncer, varias formas de los cuales se describen en la sección Definiciones más arriba. Preferiblemente, el cáncer tratado con una formulación de anticuerpo DR5 es un tumor sólido o NHL.

Donde la indicación es cáncer, el paciente puede tratarse con una combinación de la formulación de anticuerpo, y un agente quimioterapéutico. La administración combinada incluye co-administración o administración concurrente, usando formulaciones separadas o una formulación farmacéutica única, y administración consecutiva en cualquier orden, en donde preferiblemente hay un período de tiempo mientras ambos (o todos) agentes activos ejercen simultáneamente sus actividades biológicas. En consecuencia, el agente quimioterapéutico puede administrarse antes o después la administración de la composición. En esta incorporación, el mejor momento entre por lo menos una administración del agente quimioterapéutico y por lo menos una administración de la composición es preferiblemente aproximadamente 1 mes o menos y, muy preferiblemente aproximadamente 2 semanas o menos.

Alternativamente, el agente quimioterapéutico y la composición se administran concurrentemente al paciente, en una formulación única o formulaciones separadas.

El tratamiento con la formulación resultará en una mejora en los signos o síntomas de cáncer o la enfermedad. Por ejemplo, donde la enfermedad que se está tratando es cáncer, tal terapia puede resultar en una mejora en la supervivencia (supervivencia total y/o supervivencia libre de evolución) y/o puede resultar en una respuesta clínica objetiva (parcial o completa). Más aún, el tratamiento con la combinación del agente quimioterapéutico y la formulación de anticuerpo puede resultar en un beneficio terapéutico sinérgico, o más que aditivo, al paciente.

Preferiblemente, el anticuerpo en la formulación administrada es un anticuerpo desnudo. No obstante, el anticuerpo administrado puede conjugarse con un agente citotóxico. Preferiblemente, el inmunoconjugado y/o antígeno al cual se une se internaliza/internalizan por la célula, resultando en una eficacia terapéutica incrementada del inmunoconjugado en eliminar la célula cancerosa a la cual se une. En una incorporación preferida, el agente citotóxico toma como diana o interfiere el ácido nucleico en la célula cancerosa. Ejemplos de tales agentes citotóxicos incluyen maitansinoides, caliqueamicinas, ribonucleasas y ADN endonucleasas.

La formulación se administra a un paciente humano de acuerdo con métodos conocidos, tales como administración intravenosa, por ejemplo, como inyección intravenosa rápida o por infusión continua durante un período de tiempo, por las vías intramuscular, intraperitoneal, intracerobrospinal, subcutánea, intra-articular, intrasinovial, intratecal, oral, tópica, o inhalación. Se prefiere la administración intravenosa, intramuscular o subcutánea de la composición de anticuerpo, siendo muy preferida la administración intravenosa.

Para aplicación subcutánea, la formulación puede administrarse con jeringa; aparato de inyección (por ejemplo, el aparato INJECT-EASE™ y GENJECT™); pluma inyectora (tales como la GENPEN™); aparato sin

aguja (por ejemplo, MEDDECTOR™ y BIOJECTOR™); o sistema de aplicación de parche subcutáneo.

Para la prevención o tratamiento de enfermedad, la posología apropiada del anticuerpo dependerá del tipo de enfermedad a tratar, como se define anteriormente, la gravedad y ciclo de la enfermedad, si el anticuerpo se administra con propósitos preventivos o terapéuticos, la terapia previa, la historia clínica del paciente y la respuesta al anticuerpo, y la discreción del médico al cuidado del paciente. El anticuerpo se administra convenientemente al paciente de una sola vez o durante una serie de tratamientos. Dependiendo del tipo de gravedad de la enfermedad, alrededor de 1 μg/kg a 50 mg/kg (por ejemplo, 0.1-20mg/kg) de anticuerpo HER2 o DR5 es una posología candidata inicial para administración al paciente, si, por ejemplo, por una o más administraciones separadas, o por infusión continua. La posología del anticuerpo estará generalmente en el rango desde alrededor de 0.05mg/kg a alrededor de 10mg/kg. Si se administra un agente quimioterapéutico, éste se administra usualmente en posologías conocidas para él, u opcionalmente se disminuye debido a la acción combinada de los fármacos o efectos secundarios negativos atribuibles a la administración del agente quimioterapéutico. 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 también en Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

Otros regímenes terapéuticos pueden combinarse con el anticuerpo incluidos, pero no limitados a: un segundo (tercero, cuarto, etc.) agente(s) quimioterapéutico (*es decir,* "cócteles" de diferentes agentes quimioterapéuticos); otro anticuerpo monoclonal; un agente inhibidor de crecimiento; un agente citotóxico; un agente quimioterapéutico; un fármaco dirigido a EGFR; inhibidor de tirosina cinasa; un agente anti-angiogénico; y/o citocina; etc.

Aparte de los regímenes terapéuticos anteriores, el paciente puede someterse a resección quirúrgica de células cancerosas y/o terapia de radiación.

V. Artículos de Fabricación

En otra incorporación de la invención, se provee un artículo de fabricación que contiene la formulación farmacéutica de la presente invención y ofrece instrucciones para su uso. El artículo de fabricación comprende un envase. Envases apropiados incluyen, por ejemplo, frascos, frasquitos (por ejemplo, frasquitos de cámara dual), jeringas (tales como jeringas de cámara dual) y tubos de ensayo. El envase puede formarse de una variedad de materiales tales como vidrio o plástico. El envase contiene la formulación, y la etiqueta sobre, o asociaada con, el envase puede indicar instrucciones de uso. El envase que contiene la formulación puede ser un frasquito multi-uso, que permite administraciones repetidas (por ejemplo de 2-6 administraciones) de la formulación reconstituida. El artículo de fabricación puede incluir además otros materiales deseables desde un punto de vista comercial y del usuario, incluidos otros amortiguadores, diluyentes, filtros, agujas, jeringas, y prospectos de envase con instrucciones de uso como se anota en la sección anterior.

La invención se entenderá más completamente por referencia a los siguientes ejemplos. No obstante, éstos no deben interpretarse como limitantes del ámbito de la invención. Todas las citas de literatura y patentes se incorporan aquí por referencia.

EJEMPLOS

Formulaciones Líquidas Estables de Pertuzumab

Estos ejemplos describen el desarrollo y las pruebas de estabilidad de formulaciones líquidas estables que comprenden Pertuzumab en concentraciones de proteína en el rango desde alrededor de 10 mg/mL - 180

mg/mL. Las formulaciones seleccionadas tuvieron una baja turbiedad, y fueron estables física y químicamente. Se retiró un ión de cloruro de la formulación para reducir el riesgo de corrosión. La formulación era isotónica, y apropiada para aplicación subcutánea o intramuscular. La formación de agregados insolubles luego del esfuerzo de agitación se evitó usando formulación de acetato de histidina y sacarosa, sin la necesidad de incluir polisorbato 20.

Métodos Analíticos

Color, Apariencia y Claridad (CAC)

El color, apariencia y claridad de las muestras se determinaron mediante inspección visual de los frasquitos contra un fondo blanco y negro bajo luz fluorescente blanca a temperatura de la sala.

Mediciones de Concentración UV

Primero se diluyó la alícuota del producto líquido con amortiguador de formulación, de modo que la A_{max} cerca de 278 nm está en 0.5-1.0 unidades de absorbancia. La absorbancia UV de las muestras diluidas se midió en una cubeta de cuarzo con 1 cm longitud de trayectoria en un espectrofotómetro HP 8453. La absorbancia se midió a 278 nm y 320 nm. La absorbancia de 320 nm se usa para corregir la difusión de la luz de fondo, debida a agregados más grandes, burbujas y partículas. Las mediciones se calibraron contra el amortiguador de formulación. La concentración de proteína se determinó usando la absortividad de 1.50 $(mg/mL)^{-1}cm^{-1}$.

Mediciones de pH

Se midió el pH a temperatura de la sala usando un medidor de pH RADIOMETER COPENHAGEN PHM82™. La sonda usada fue un electrodo de referencia/vidrio combinado con un conector radiometer (Sigma, Cat# E-5759). Se usaron soluciones estándar de pH 4.01 y pH 7.00 (EM Science) para calibración del medidor de pH.

Cromatografía de Intercambio Iónico (IEX)

Se empleó cromatografía de intercambio catiónico para medir variaciones en variantes de carga. Este análisis utiliza una columna DIONEX PROPAC WCX-10™ en un sistema HPLC HP 1100™. Las muestras se diluyeron a 1 mg/mL con la fase móvil A que contenía 20 mM MES a pH 6.0. Luego se cargaron 50 mL de muestras diluidas en la columna que se mantuvo a temperatura ambiente. Los picos se eluyeron con una gradiente NaCl superficial usando fase móvil B que contenía 20 mM MES, 250 mM NaCl, pH 6.0. El eluyente se monitoreó a 280 nm. Los datos se analizaron usando software HP CHEMSTATION™ (Rev A08.03).

Electroforesis de Zona Capilar (CZE)

La pureza de los fragmentos Fab y $F(ab')_2$ se determinó por CZE. Este análisis se ejecutó en un sistema de electroforesis capilar BIORAD BIOFOCUS™ 3000™ con un capilar BIOCAP XL™, 50 µm I.D., longitud total 44.6 cm y 40 cm al detector.

Cromatografía de Exclusión de Tamaño (SEC)

Se utilizó cromatografía de exclusión de tamaño para cuantificar agregados y fragmentos. Este análisis utiliza una columna TSK G3000 SWXL™, 7.8 x 300 mm y se ejecuta en un sistema HPLC HP 1100™. Se diluyeron muestras a 10 mg/mL con la fase móvil y el volumen de inyección fue de 20 µL. La fase móvil fue 100 mM K_2HPO_4 a pH 6.8 y la proteína se eluyó con una gradiente isocrática a 0.5 mL/minuto durante 45 minutos. Se monitoreó la absorbancia del eluyente a 280 nm. La integración se realizó usando software HP CHEMSTATION™ (Rev. A08.03).

Actividad Biológica

Se determinó la actividad biológica de Pertuzumab midiendo su capacidad de inhibir la proliferación de la línea de células de cáncer de mama humano MDA-MB-175-VII.

EJEMPLO 1

Fragmentos de anticuerpo Fab y $F(ab')_2$ Pertuzumab se formularon con una concentración de proteína de 1.0 mg/mL en las siguientes condiciones de amortiguador:

10 mM citrato, 140 mM NaCl, pH 4.0;

10 mM succinato, 140 mM NaCl, pH 5.0;

10 mM succinato, 140 mM NaCl, pH 6.0;

10 mM histidina, 140 mM NaCl, pH 7.0; y

10 mM glicilglicina, 140 mM NaCl, pH 8.0.

Cada formulación se filtró, luego se repartió en alícuotas en frasquitos de vidrio tipo I WHEATON™ USP de 3 cc sellados con tapones de butilo gris recubiertos con TEFLON™. Las muestras se almacenaron a 40 ± 2 °C. Los análisis de estabilidad de producto farmacéutico mostraron que los Fab y $F(ab')_2$ fueron más estables entre pH 5.0 y 6.0.

Tabla 2. Efecto del pH sobre la degradación de Fab o $F(ab')_2$ almacenadas a 40°C

pH de la formulación	Fab		$F(ab')_2$	
	CZE % Pico Principal	CZE % Pico Principal	CZE % Pico Principal	CZE % Pico Principal
4.0	74.1	96.7	43.6	89.4
5.0	83.2	96.4	65.4	94.0
6.0	82.9	96.2	69.0	92.3

7.0	83.9	96.4	62.3	91.3
8.0	72.7	96.4	49.2	89.8

EJEMPLO 2

Pertuzumab se formuló en 20 mM de amortiguador de acetato de histidina con 120 mM sacarosa y 0.02 % polisorbato 20. Los pH de las formulaciones se ajustaron con ácido acético a un pH final entre 5.0 y 7.0. La concentración de proteína fue de 30 mg/mL. Cada formulación se envasó en frasquitos de vidrio tipo I USP de 3 cc y se almacenaron a 40°C para análisis de estabilidad. Los resultados mostraron que Pertuzumab fue muy estable alrededor de pH 6.0.

Tabla 3. Efecto del pH sobre la degradación de Pertuzumab almacenado a 40°C

pH de la Formulación	Temperatura (°C)	Tiempo de Almacenamiento (semanas)	SEC % Monómero	IEX % Pico Principal
5.0	40	2	99.4	57.4
5.5	40	2	99.4	59.2
6.0	40	2	99.4	60.6
6.5	40	2	99.3	60.5
7.0	40	2	99.1	54.0
5.0	40	4	97.3	48.1
5.5	40	4	99.1	50.5
6.0	40	4	99.1	53.3
6.5	40	4	99.0	52.3
7.0	40	4	98.6	42.3

EJEMPLO 3

Se prepararon formulaciones de Pertuzumab con una concentración de proteína de 100 mg/mL en los siguientes excipientes:

295

(1) 10 mM histidina-HCl, 240 mM sacarosa, 0.02% polisorbato 20, pH 6.0;
(2) 10 mM acetato de histidina, 240 mM sacarosa, 0.02% polisorbato 20, pH 6.0;
(3) 10 mM histidina-fosfato, 240mM sacarosa, 0.02% polisorbato 20, pH 6.0;
(4) 10 mM histidina-sulfato, 240 mM sacarosa, 0.02% polisorbato 20 a pH 6.0.

Cada formulación se envasó en frasquitos de vidrio tipo I FORMA VITRUM™ USP WHEATON™ USP de 3 cc sellados con tapones de caucho butilo recubiertos con FLUROTEC™. Se almacenaron muestras a 30°C y 40°C, y se evaluó la estabilidad en cuanto a la calidad (CAC) y pureza (SEC, IEC). Los resultados de estabilidad mostraron que Pertuzumab en amortiguador de histidina-fosfato se degradó mucho más rápido en otros amortiguadores de histidina luego del almacenamiento a 40°C (Fig. 8 y Fig. 9).

EJEMPLO 4

Pertuzumab se concentró por ultrafiltración/diafiltración a varias concentraciones en los siguientes amortiguadores:
(1) 20 mM acetato de histidina, pH 6.0;
(2) 10 mM histidina-HCl, pH 6.0, y
(3) 10 mM histidina-sulfato, pH 6.0.

La turbiedad de cada formulación se midió antes de la filtración. Los resultados, como se ilustran en Fig. 10, demostraron que muestras de Pertuzumab formuladas en acetato de histidina e histidina-HCl tuvieron menor cantidad de agregados insolubles que aquellos en amortiguador histidina-sulfato.

EJEMPLO 5

Pertuzumab se formuló a 30 mg/mL en 20 mM acetato de histidina, 120 mM sacarosa, 0.02 % polisorbato 20, pH 6.0. El Pertuzmab se envasó en recipientes miniatura de acero inoxidable 316L y HASTELLOY™. Todas las muestras se almacenaron a -20°C y 5°C y se evaluaron con respecto a la calidad (CAC), pureza (SEC, IEC) y consistencia (UV-Vis). Los análisis de estabilidad mostraron que Pertuzumab era estable en esta formulación luego del almacenamiento a -20°C y 5°C durante por lo menos 3 meses. La formulación libre de cloruro es compatible con el recipiente de acero inoxidable 316L y HASTELLOY™.

Tabla 4. Estabilidad de Pertuzumab en Recipientes de Acero Inoxidable

Recipientes	Temp. (°C)	Tiempo (Meses)	CAC	UV Spec. (mg/mL)	SEC (% Monómero)	IEC (% Pico Principal)
		0	Pass[a]	29.0	99.8	67.9
316L	-20	3	Pass	28.9	99.7	66.8
	5	3	Pass	28.7	99.7	66.8
HASTELLON™	-20	3	Pass	29.1	99.7	66.8
	5	3	Pass	28.8	99.7	67.7

[a] Aprobación para Color, Apariencia y Claridad: Solución clara a ligeramente opalescente, incolora a amarillo pálido.

EJEMPLO 6

Pertuzumab se formuló usando filtración de flujo tangencial (TFF). La formulación final contiene 20 mM de acetato de histidina, 120 mM sacarosa, 0.02 % polisorbato 20, pH 6.0 con una concentración de proteína de 30 mg/mL. Las muestras se envasaron en un frasquito de vidrio tipo I FORMA VITRUM™ USP de 20 Ml, tapados con los tapones de caucho de

butilo 20 mm recubiertos con FLUROTEC™, y se sellaron con tapas flip-top de aluminio. Todas las muestras se almacenaron a -70°C, 5°C, 15°C, y se evaluó la estabilidad con respecto a la calidad (CAC), pureza (SEC, IEC), consistencia (UV- Vis), y potencia (Bioanálisis). Los resultados mostraron que Pertuzumab es estable en esta formulación luego del almacenamiento a 5°C y 15°C durante por lo menos 3 meses.

Tabla 5. Estabilidad de Pertuzumab en frasquitos de vidrio

Temperatura (°C)	Tiempo (Meses)	CAC	UV Spec. (mg/mL)	SEC (% Monómero)	IEC (% Pico Principal)	Bioanálisis (% Actividad específica)
	0	Pass	29.2	99.8	64.1	83
-70	1	Pass	29.7	99.8	65.2	92
	3	Pass	30.7	99.8	67.0	93
5	3	Pass	30.4	99.7	67.2	90
15	1	Pass	29.7	99.7	64.4	78
	3	Pass	30.4	99.7	65.5	93

EJEMPLO 7

Pertuzumab se formuló a 100 mg/mL en las siguientes condiciones de amortiguador:

(1) 10 mM histidina-HCl, pH 6.0;

(2) 10 mM histidina-HCl, 240 mM sacarosa, pH 6.0;

(3) 20 mM succinato a pH 6.0; y

(4) 20 mM succinato, 240 mM sacarosa a pH 6.0.

Cada formulación se agregó con diferente concentración de polisorbato 20. Todas las muestras se envasaron en frasquitos de vidrio tipo I USP de 3 cc y se agitaron horizontalmente a 70 rpm a temperatura de la sala durante hasta 7 días. La estabilidad de cada muestra se evaluó en el punto de tiempo correspondiente al día 7 con respecto a la turbiedad. Los resultados demostraron que el uso de polisorbato 20 en la formulación final

impidió efectivamente la formación de agregados insolubles. Véase Fig. 11.

EJEMPLO 8

Pertuzumab se preparó en las siguientes formulaciones:

(1) 25 mg/mL Pertuzumab, 10 mM histidina-HCl, 240 mM sacarosa, pH 6.0;

(2) 50 mg/mL Pertuzumab, 10 mM histidina-HCl, 240 mM sacarosa, pH 6.0;

(3) 60 mg/mL Pertuzumab, 20 mM acetato de histidina, 120 mM sacarosa, pH 6.0.

Varias cantidades de polisorbato 20 se agregaron a cada formulación. Todas las muestras se envasaron en frasquitos de vidrio tipo I USP de 3 cc, y se agitaron horizontalmente a 70 rpm a temperatura de la sala durante hasta 7 días. Se evaluó la estabilidad física de cada muestra en el punto de tiempo correspondiente al día 7 con respecto a turbiedad. Los resultados demostraron que el uso de polisorbato 20 en formulación de histidina-HCl y sacarosa evitó efectivamente la formación de particulados insolubles. La formulación que contenía acetato de histidina y sacarosa parecieron tener el mismo efecto protector sobre la proteína, que el polisorbato 20. Véase Fig. 12.

EJEMPLO 9

Pertuzumab se formuló en la siguiente forma:

(1) 100 mg/mL proteína, 10 mM histidina-HCl, pH 6.0;

(2) 100 mg/mL proteína, 20 mM succinato, pH 6.0;

(3) 60 mg/mL proteína, 20 mM acetato de histidina, pH 6.0.

Cada formulación se mezcló con diferentes cantidades de sacarosa. Todas las muestras se envasaron esterilizadamente en frasquitos de vidrio tipo I USP de 3 cc. Éstos se congelaron entonces a -70°C y se descongelaron a 5°C tres veces. La estabilidad física de cada muestra se determinó luego de los tres ciclos de congelamiento y descongelamiento.

Los resultados demostraron que la sacarosa evita la formación de agregados solubles durante el proceso de congelamiento-descongelamiento. Véase Fig. 13.

EJEMPLO 10

La formulación preferida de Pertuzumab para uso terapéutico consiste esencialmente de 30mg/mL Pertuzumab en 20mM de acetato de histidina, 120mM sacarosa, 0.02% polisorbato 20, con un pH 6.0.

Compuesto	Concentración	Cantidad/L
Pertuzumab	30 mg/mL	30 g
L-Histidina MW=155.16g/mol	20 mM	3.10 g
Ácido Acético Glacial MW=60.05g/mol Densidad=1.05g/cm^3	11.6 mM	0.66 mL
Sacarosa MW=342.3g/mol	120 mM	41.1 g
Polisorbato 20 Densidad=1.012g/cm^3	0.02% (p/v)	0.2 mL

MW: Peso Molecular

Configuración de frasquito con una dosis de 420mg:

Frasquito: vidrio tipo I Formal Vitrum de 20 cc

Tapón: 20mm DAIKYO GREY™, laminado con fluoro-resina

Tapa: 20mm aluminio flip top

Volumen de envasado: 14.50 mL

Aplicación: 14.0 mL Pertuzumab en bolsa de solución salina normal IV.

Configuración de frasquito con una dosis de 1.050mg:

Frasquito: vidrio tipo Formal Vitrum de 50 cc

Tapón: 20mm DAIKYO GREY™, laminado con fluoro-resina

Tapa: 20mm aluminio flip top

Volumen de envasado: 36.0 mL

Aplicación: 35.0 mL Pertuzumab e bolsa de solución salina normal IV.

EJEMPLO 11

Este ejemplo concierne a otra formulación de Pertuzumab que se ha usado en estudios clínicos de Fase I y Fase II. La composición consiste de 25 mg/ml Pertuzumab, 10 mM de amortiguador Histidina-HCl, 240 mM sacarosa, 0.02% Polisorbato 20, pH 6.0.

EJEMPLO 12

La apoptosis celular es mediada por vías intrínsecas y extrínsecas. La quimioterapia puede causar lesión a las células y desencadenar apoptosis por la vía intrínseca en respuesta a lesión celular. Sin embargo, a menudo las células cancerosas desarrollan resistencia a la quimioterapia a través de mutaciones en el gen supresor de tumores p53 (Ashkenazi A. Targeting Death and Decoy Receptors of the Tumour-Necrosis Factor Superfamily. *Nature Reviews* 2:420- 430 (2002)). Los receptores de muerte, tales como DR4 y DR5, localizados en la superficie de células, desencadenan apoptosis mediante la vía extrínseca que no involucra p53. Moléculas agonísticas, tales como Apo2L, se unen a receptores DR4 y DR5 y activan las caspasas 8 y 10 a través del dominio de muerte asociado con Fas. Las caspasas 8 y 10 activan luego las caspasas 3, 6, y 7 para inducir apoptosis. La señalización molecular de receptores de muerte sobre las células tumorales tiene un potencial terapéutico para la eliminación de células cancerosas que son resistentes a terapias convencionales; y moléculas, como Apo2L, están experimentando en la actualidad evaluación clínica.

"Apomab" es una IgG1 humana derivada de CHO de longitud total, construida con una cadena ligera λ. Es un anticuerpo agonista contra DR5 que ha demostrado que induce apoptosis de varias líneas de células cancerosas. Estudios preclínicos usando un modelo de implante de tumor murino han demostrado que Apomab posee una reducción del tumor

mejorada o similar, comparado con Apo2L. Apomab está siendo evaluado como agente anti-cáncer en las indicaciones de tumores sólidos avanzados y Linfoma no Hodgkiniano (NHL). Las secuencias de aminoácidos de cadena ligera y pesada de Apomab usadas en estos experimentos se muestran en Figs. 27 y 28.

Preparación de Formulaciones de Anticuerpo

Apomab producido recombinantemente tuvo una concentración de proteína diluida y pH altos. El material se concentró a aproximadamente 20 mg/mL y se intercambió en 20 mM de amortiguador de acetato de sodio, pH 5.0 usando un sistema de filtración de flujo tangencial (TFF) Millipore Labscale con membrana MILLIPORE PELLICON™ XL, PLCGC10, 50 cm. Muestras de Apomab se formularon en varios sistemas amortiguadores que cubrían un rango de pH desde 4.0 a 7.0 usando acetato de sodio, acetato de histidina, y fosfato de sodio sin trehalosa y TWEEN 20® usando diálisis con una membrana de separación de 10.000 Da de peso molecular (Pierce, Inc). En la última diálisis se agregó trehalosa a 240 mM. Luego de la diálisis, se agregó 0.02% TWEEN 20™ a la formulación, y las muestras se filtraron con filtros de 0.22 μm (Millipore, Inc.). Un volumen de 0.5 mL de Apomab se envasó en frasquitos de vidrio esterilizados de 3 cc (Forma Vitrum, Inc.) y se selló con tapones de 13 mm (Daikyo, Inc). Se evaluó la estabilidad de la proteína a -70°C, 5°C, 30°C, y 40°C con almacenamiento durante hasta 3 meses.

Estabilidad de la Formulación de Apomab

Para las pruebas de estabilidad del producto farmacéutico, Apomab formulado a granel se envasó en frasquitos de vidrio FORMA VITRUM® de 5 cc. Los frasquitos se envasaron con 5.5 mL del anticuerpo formulado, equipados con tapones DAIKYO® de 20 mm, y se almacenaron a -70°C, 5°C, 30°C, y 40°C en la posición vertical.

Para las pruebas de estabilidad de la sustancia farmacéutica, Apomab formulado a granel se filtró esterilizadamente a través de un filtro de 0.22

μm, y 10 mL se envasaron en mini-recipientes de acero inoxidable 316L de 20 cc autoclavados. Los recipientes se pusieron en posición vertical a -20°C y 5°C. Se retiró asépticamente una alícuota de 1 mL de los mini-recipientes en intervalos especificados de tiempo para evaluar la calidad de la proteína. Los frasquitos de control eran alícuotas de 1 mL en frasquitos de vidrio de 3 cc almacenados a -20°C.

Color, Apariencia, y Claridad

La claridad, apariencia, y color de las muestras se evaluaron visualmente bajo luz fluorescente blanca usando una estación de inspección lumínica con fondo blanco y negro. Para análisis de la sustancia farmacéutica, se transfirieron muestras de los mini-recipientes a un frasquito de vidrio de 3 cc para inspección.

PH

El pH se midió a temperatura de la sala con un electrodo semi-micro de pH THERMO ORION SURE-FLOW ROSS™ para medir amortiguadores o electrodo micro de pH de combinación THERMO ORION GLS™ para medir muestras de cribado de pH de proteína, una sonda de microelectrodo Beckman para muestras de estabilidad en Toxicología. El medidor METERLAB™ pHM240 pH/Ion (Radiometer Analytical) se calibró cada día con estándares de amortiguador (EM Science) a pH 7 y pH 4.

Concentración

La concentración de proteína se determinó por espectroscopía de absorción ultravioleta usando un espectrofotómetro AGILENT 8453™. Las muestras se diluyeron con blancos de amortiguadores de formulación apropiada para producir una absorbancia desde 0.5 a 1.0. El instrumento se calibró con la solución diluyente y el espectro se barrió desde 240 a 500 nm. El valor de absorbancia a 320 nm se restó de la absorbancia a 279 nm

para corregir la desviación y difusión de la luz. Las concentraciones de proteína se calcularon por la siguiente ecuación:

$$\text{Conc. (mg/mL)} = \frac{(A279 - A320) \times \text{factor de dilución}}{\text{coeficiente de absortividad en } cm^{-1}mg/mL)^{-1}}$$

Inicialmente se determinó que el coeficiente de absortividad basado en la secuencia era 1.32 cm^{-1} $(mg/mL)^{-1}$ y este valor se utilizó para los estudios de cribado de pH. Un valor posterior de 1.7 $cm^{-1}(mg/mL)^{-1}$ se determinó mediante análisis de aminoácidos y métodos de proteólisis, y este valor se empleó para el análisis de estabilidad de Apomab usado en estudios de Toxicología.

Cromatografía de Intercambio Iónico

Se llevó a cabo cromatografía de intercambio iónico en HPLC serie 1100 (Agilent Technologies, Inc.), equipada con un detector de arreglo de díodo. La cromatografía se realizó en una columna PROPAC WCX-10™ (Dionex) (4 x 250 mm) con un flujo de 0.5 mL/minuto y con una temperatura de la columna a 40°C. La fase móvil A fue 25 mM de fosfato de sodio, pH 6.5. La fase móvil B fue 100 mM de cloruro de sodio en el mismo amortiguador que la fase móvil A. La columna se equilibró con 100% de fase móvil A. Para muestras de cribado de pH se cargó una cantidad de 20 mg de Apomab en la columna, y se monitoreó la absorbancia a 214 nm. La proteína se eluyó de la columna con la siguiente gradiente:

Tiempo (min)	% A	% B
0	100	0
50	0	100
51	100	0
70	100	0

Para el análisis de estabilidad del material utilizado en los estudios de Toxicología se cargó una cantidad de 30 mg de Apomab en la columna, y se

monitoreó la absorbancia a 280 nm. La proteína se eluyó de la columna con la siguiente gradiente:

Gradiente:	Tiempo (min)	% A	% B
	0	100	0
	40.0	40	60
	41.0	0	100
	45.0	0	100
	45.1	100	0
	60.0	100	0

Cromatografía de Exclusión de Tamaño

La cromatografía de exclusión de tamaño se llevó a cabo en una HPLC serie 1100 (Agilent Technologies, Inc.), equipada con un detector de arreglo de díodo. Una cantidad de 50 µg Apomab se cargó en una columna TSK Gel 3000SWXL™ (7.8 x 300 mm) y se ejecutó con un caudal de 0.9 mL/minuto durante 20 minutos para muestras de cribado de pH y 0.5 mL/minuto durante 30 para muestras de estabilidad en Toxicología con 0.20 M de fosfato de potasio, 0.25 M de cloruro de potasio, pH 6.2 como fase móvil. La absorbancia de monitoreó a 280 nm.

Potencia

El propósito del bioanálisis de potencia era medir la capacidad de Apomab de eliminar células Colo205 usando ALAMARBLUE™. Colo205 es una línea de células de carcinoma del colon, que expresa los dos receptores de muerte DR5 y DR4. Este análisis incorpora un indicador de crecimiento fluorométrico/colorimétrico basado en la detección de actividad metabólica. ALAMARBLUE™ es un tinte redox que es azul y no fluorescente en estado oxidado. La reducción metabólica intracelular lo convierte en un color rojo que es también fluorescente. Los cambios en color y fluorescencia son proporcionales a la actividad metabólica y al número de células vivas. La señal decreció al morir las células. Apomab se

diluyó en medio con anti-Fc, y luego se agregaron las células Colo205 a las muestras de Apomab y se incubó a 37°C durante 48 horas. Se agregó ALAMARBLUE™ durante las últimas 2-3 horas. Se leyó la placa a 530 nm de excitación y 590 nm de emisión para obtener unidades de fluorescencia relativa (RFU). Los datos se analizaron mediante KALEIDAGRAPH™. Se generó una curva de dilución de la eliminación.

RESULTADOS

Estudio de Cribado de pH en la Formulación

Se estudió el efecto del pH sobre la estabilidad del anticuerpo usando Apomab producido de una línea de células estable no amplificada. Para este análisis, se formuló Apomab con 20 mg/mL de anticuerpo en un amortiguador de 20 mM de acetato de sodio con pH 4.0, 4.5, 5.0, 5.5; 20 mM de amortiguador de acetato de histidina con pH 6.0 y 6.5; y 20 mM de amortiguador de fosfato de sodio con pH 7.0. Todas estas formulaciones contenían 240 mM de trehalosa y 0.02% TWEEN 20®. Las formulaciones se almacenaron durante hasta 3 meses a temperaturas de -70°C, 5°C, 30°C, y 40°C y se determinó la estabilidad de la proteína mediante varias pruebas analíticas, incluidas CAC, pH, concentración, SEC e IEC. No se observaron cambios significativos en CAC, pH o concentración de proteína durante el almacenamiento de las muestras.

El análisis de las muestras por SEC mostró que no ocurrieron cambios significativos durante el almacenamiento a 5°C y -70°C. No obstante, la degradación observada como la formación de fragmentos de anticuerpo y agregados solubles ocurrió durante el almacenamiento a 30°C y 40°C (Fig. 20). Para comparar las formulaciones se monitoreó la cinética del monómero de anticuerpo durante el almacenamiento y se calcularon las constantes de tasa de primer orden. El perfil de la tasa de pH obtenida para la pérdida en monómero de anticuerpo se muestra en Fig. 21. La condición óptima para la estabilidad del monómero de anticuerpo se obtuvo formulando un amortiguador de acetato de histidina con pH 6.0.

La heterogeneidad de carga del Apomab se monitoreó por IEC. No ocurrieron cambios significativos en el perfil IEC durante el almacenamiento a 5°C y -70°C. No obstante, la degradación observada como la formación de variantes ácidas o básicas ocurrió dependiendo de la formulación (Fig. 22). En general, se formaron variantes básicas incrementadas con un pH de formulación menor y variantes más ácidas se formaron con un pH de formulación más elevado. Para comparar las formulaciones, se monitoreó la cinética de pico principal IEC durante el almacenamiento y se calcularon las constantes de tasa de primer orden. El perfil de tasa de pH obtenido para la pérdida en el pico principal IEC se muestra en Fig. 23. Las constantes de tasa observadas por IEC fueron aproximadamente 10 veces más altas que las de SEC (Fig. 21). En consecuencia, la pérdida en pico principal IEC fue la degradación primaria del anticuerpo que finalmente limitará la vida útil del producto. Además, como se observó por SEC, la estabilidad de anticuerpo óptima para estabilizar el pico principal IEC se obtuvo formulando en amortiguador de acetato de histidina con pH 6.0.

Luego del análisis de los datos de cribado de pH ya descritos, se seleccionó una formulación de Apomab que comprendía 20 mg/mL de anticuerpo en 20 mM de acetato de histidina, 240 mM de trehalosa, 0.02% polisorbato 20, pH 6.0. Para el producto farmacéutico, la configuración de frasquito consistió de 5.5 mL de carga en un frasquito FORMA VITRUM™ de 5 cc con un tapón 20 mM DAIKYO™ West. El Apomab se almacenó en recipientes de acero inoxidable.

La estabilidad del Producto Farmacéutico Apomab se evaluó en la configuración de frasquito de vidrio de 5cc descrito anteriormente. Los frasquitos se almacenaron a -70°C (controles), 5°C, 30°C, y 40°C. Las muestras se extrajeron en intervalos de tiempo específicos y se analizaron mediante las siguientes pruebas: color, apariencia, claridad (CAC), pH, concentración de proteína, SEC, IEC, y potencia. Los resultados de estos análisis se muestran en la Tabla 6 para muestras almacenadas a -70°C y 5°C y la Tabla 7 para las muestras almacenadas a 30°C y 40°C.

Tabla 6. Datos de Estabilidad para Apomab Almacenado a -70°C y 5°C

Temperatura (°C)	Punto de Tiempo	Claridad	Color	pH	Concentración (mg/mL)	SEC (% Monómero)	IEC (% Pico Principal)	Potencia (% Actividad Específica)
Criterios de	*Aceptación:*	*Informe*	*Informe*	*6.0±0.3*	*20±2*	*≥95%*	*Informe*	*60 – 140%*
NA	T=0	Claro	Incoloro	5.9	20.2	99.8	63	94
-70	1 mes	Claro	Incoloro	6.0	20.5	99.8	63	86
-70	2 meses	Claro	Incoloro	6.0	20.4	99.7	64	91
-70	3 meses	Claro	Incoloro	6.0	20.5	99.7	63	83
-70	6 meses	Claro	Incoloro	6.0	20.4	99.7	64	85
-70	9 meses	Claro	Incoloro	6.0	20.4	99.8	65	89
-70	12	Claro	Incoloro	6.0	20.8	99.7	63	107
5	mes	Claro	Incoloro	6.0	20.5	99.7	63	89
5	1 mes	Claro	Incoloro	6.0	20.4	99.7	64	99
5	2 meses	Claro	Incoloro	6.0	20.6	99.7	63	84
5	6 meses	Claro	Incoloro	6.0	20.5	99.7	64	93
5	9 meses	Claro	Incoloro	6.0	20.6	99.7	64	88
5	12 meses	Claro	Incoloro	6.0	20.7	99.6	64	106

Tabla 7. Datos de Estabilidad para Apomab Almacenado a 30°C y 40°C

Temperatura (°C)	Punto de Tiempo	Claridad	Color	pH	Concentración (mg/mL)	SEC (% Monómero)	IEC (% Pico Principal)	Potencia (% Actividad Específica)
Criterios de	*Aceptación:*	*Informe*	*Informe*	*6.0±0.3*	*20±2*	*≥95%*	*Informe*	*60 – 140%*
30	1 mes	Claro	Incoloro	6.0	20.6	98.2	59	91
30	2 meses	Claro	Incoloro	6.0	20.3	97.4	54	80
30	3 meses	Claro	Incoloro	6.0	20.6	97.2	49	74
30	6 meses	Claro	Incoloro	6.0	20.2	94.1	37	51
30	9 meses	Claro	Ligeramente amarillo	6.0	20.4	93.2	31	55
30	12 meses	Claro	Ligeramente amarillo	6.0	20.6	91.6	25	59
40	1 meses	Claro	Incolora	6.0	20.4	96.6	44	79
40	2 meses	Claro	Incolora	6.0	20.0	93.7	31	64
40	3 meses	Claro	Ligeramente amarillo	5.9	20.3	91.5	22	53
40	6 meses	Claro	Ligeramente amarillo	6.0	20.2	83.9	NT	26
40	9 meses	Claro	Amarillo	5.9	20.3	78.8	NT	25
40	12 meses	Claro	Amarillo	5.9	20.5	71.4	NT	31

NT = no cuantificado

No se observó cambio en la calidad de proteína después de doce meses de almacenamiento a -70°C y 5°C. Por ejemplo, el pH permaneció a 6.0 ± 0.3, el Apomab apareció como un líquido claro e incoloro, la concentración de proteína permaneció a 20.0 ± 2.0 mg/mL, y el % de monómero no varió. Más aún, no hubo cambio significativo en el % pico principal IEC, y el % actividad específica determinada por el análisis de potencia de eliminación celular estuvo dentro de la precisión del análisis de 60 % a 140 % de actividad específica. Los resultados mostraron que el Apomab almacenado en frasquitos de 5 cc era estable durante por lo menos 12 meses a 5°C.

Tabla 7 muestra que ocurrieron cambios en la calidad de la proteína a 30°C y 40°C. La SEC mostró una disminución en el % de monómero con un aumento principalmente en la especie fragmento. Los agregados se incrementan también a temperatura más elevada, pero la tasa fue mucho más lenta. Sin embargo, los agregados aumentan significativamente después de 6 meses a 40°C. El pico principal % IEC disminuyó con un incremento correspondiente en las variantes ácidas. Los picos básicos disminuyeron ligeramente después de 2 meses a 40°C y 9 meses a 30°C. Luego de seis meses de almacenamiento a 40°C, ocurrió degradación en una medida en que el pico principal IEC ya no pudo integrarse. El bioanálisis de eliminación de células mostró pérdida de % de actividad específica a temperatura más elevada con un tiempo de almacenamiento más prolongado. La concentración de proteína y el pH permanecieron sin modificación. La solución se vuelve ligeramente amarilla después de 3 meses a 40°C y 9 meses a 30°C, y se torna amarilla después de 9 meses a 40°C.

Estabilidad de la Sustancia Farmacéutica

Los datos de estabilidad congelamiento-descongelamiento para la sustancia farmacéutica se muestran en la Tabla 8.

Tabla 8. Datos de Estabilidad Congelamiento-Descongelamiento para Apomab Envasado en Recipientes Miniatura de Acero Inoxidable

Temperatura (°C) (Cong./Descong.)	Ciclo Cong.-Descong. No.	Claridad	Color	pH	Concentración (mg/m L)	SEC (% Monómero)
Criterios de Aceptación:		*Informe*	*Informe*	*6.0±0.3*	*20.0±2.0*	*>95%*
Control (no congelado)	0	Clara	Incolora	6.0	20.9	99.6
-20/25	1	Clara	Incolora	6.0	20.8	.99.6
-20/25	2	Clara	Incolora	6.0	20.8	99.6
-20/25	3	Clara	Incolora	6.0	20.9	99.6

Tabla 9. Datos de Estabilidad para Apomab Envasado en Recipientes Miniatura de Acero Inoxidable

Temperatura (°C)	Punto de Tiempo	Claridad	Color	pH	Concentración (mg/mL)	SEC (% Monómero)	IEC (% Pico Principal)	Potencia (% Actividad Específica)
Criterios de	*Aceptación:*	*Informe*	*Informe*	*6.0±0.3*	*20±2*	*≥ 95%*	*Informe*	*60 – 140%*
NA	T=0	Clara	Incolora	5.9	20.0	99.7	63	88
-20	1 meses	Clara	Incolora	6.0	20.6	99.7	63	107
-20	3 meses	Clara	Incolora	6.0	20.6	99.7	63	82
-20	6 meses	Clara	Incolora	6.0	20.3	99.7	64	92
-20	9 meses	Clara	Incolora	6.0	20.6	99.7	64	92
-20	12 meses	Clara	Incolora	6.0	21.2	99.7	65	94
5	1 meses	Clara	Incolora	6.0-	20.5	99.7	62	95
5	3 meses	Clara	Incolora	6.0	20.7	99.6	62	71
5	6 meses	Clara	Incolora	6.0	20.4	99.5	62	84
5	9 meses	Clara	Incolora	6.0	20.8	99.4	61	84
5	12 meses	Clara	Incolora	6.0	21.3	99.2	59	82

No se observaron cambios significativos en las características químicas de la proteína luego de congelarse a -20°C durante por lo menos 15 horas y descongelarse a temperatura ambiente tres veces. Por ejemplo, Apomab apareció como un líquido claro e incoloro, el pH permaneció en 6.0 ± 0.3, y el porcentaje de pico de monómero SEC no experimentó modificación.

La estabilidad del Apomab en recipientes de acero inoxidable se evaluó a -20°C y 5°C (Tabla 9).

Las muestras se extrajeron asépticamente de los mini-recipientes en intervalos específicos y se analizaron.

Apomab no mostró cambio en la calidad de la proteína a 5°C ocasionado por pH, CAC, concentración de la proteína y pico principal % por IEC pero perdió 0.1% de monómero por SEC cada 3 meses. Se observó potencia disminuída durante el almacenamiento a 5°C durante 3 meses. Sin embargo, la potencia de la muestra se incrementó nuevamente en los puntos de tiempo 6 y 9 meses. Por lo tanto, la diferencia de potencia observada en el punto de tiempo 3 meses se atribuyó a la variación del análisis. Apomab no mostró cambio en la calidad de la proteína a -20°C por pH, CAC, concentración de proteína, % de monómero por SEC, pico principal % por IEC, y ningún cambio significativo en potencia. Los datos de estabilidad muestran que Apomab es estable durante por lo menos 1 año a - 20°C y tres meses a 5°C.

CONCLUSIÓN

Se realizaron estudios de cribado de la formulación para seleccionar una formulación para Apomab. Un cribado de pH que cubría el rango de pH 4.0 a 7.0 usando acetato de sodio, acetato de histidina, y fosfato de sodio como amortiguadores con 240 mM de dihidrato de trehalosa y 0.02% polisorbato 20 mostró que Apomab en muy estable en solución a pH 6.0. En consecuencia, una formulación compuesta por 20 mM de acetato de histidina, 240 mM de trehalosa, 0.02% polisorbato 2, pH 6.0 se desarrolló y demostró en los experimentos que era estable. Usando esta formulación, Apomab demostró ser estable durante por lo menos 12 meses a 5°C. Más aún, Apomab demostró ser estable durante por lo menos 12 meses a - 20°C y tres meses a 5°C cuando se almacenó en recipientes de acero inoxidable 316L. Además Apomab demostró ser estable cuando se sometió hasta a 3 ciclos de congelamiento/descongelamiento.

LO QUE SE REIVINDICA ES:

1. Una formulación farmacéutica estable que comprende un anticuerpo monoclonal en un amortiguador de acetato de histidina, pH 5.5 a 6.5.

2. La formulación de la reivindicación 1 en donde el pH es desde 5.8 a 6.2.

3. La formulación de la reivindicación 1 en donde la concentración del amortiguador de acetato de histidina es desde alrededor de 1mM a alrededor de 200mM.

4. La formulación de la reivindicación 3 en donde la concentración del amortiguador de acetato de histidina es desde alrededor de 10mM a alrededor de 40mM.

5. La formulación de la reivindicación 1 en donde la concentración de anticuerpo es desde alrededor de 10mg/mL a alrededor de 250mg/mL.

6. La formulación de la reivindicación 5 en donde la concentración del anticuerpo monoclonal es desde alrededor de 20mg/mL a alrededor de 40mg/mL.

7. La formulación de la reivindicación 5 en donde la concentración del anticuerpo monoclonal es desde alrededor de 80mg/mL a alrededor de 250mg/mL.

8. La formulación de la reivindicación 1 que comprende además un sacárido.

9. La formulación de la reivindicación 8 en donde el sacárido es un disacárido.

10. La formulación de la reivindicación 8 en donde el sacárido es trehalosa.

11. La formulación de la reivindicación 8 en donde el sacárido es sacarosa.

12. La formulación de la reivindicación 8 en donde la concentración de sacárido es desde alrededor de 10mM a alrededor de 1M.

13. La formulación de la reivindicación 12 en donde la concentración de sacárido es desde alrededor de 60mM a alrededor de 250mM.

14. La formulación de la reivindicación 1 que comprende además un surfactante.

15. La formulación de la reivindicación 14 en donde el surfactante es polisorbato.

16. La formulación de la reivindicación 15 en donde el surfactante es polisorbato 20.

17. La formulación de la reivindicación 14 en donde la concentración de surfactante es desde alrededor de 0.0001% a alrededor de 1.0%.

18. La formulación de la reivindicación 17 en donde la concentración de surfactante es desde alrededor de 0.01% a alrededor de 0.1%.

19. La formulación de la reivindicación 1 en donde el anticuerpo monoclonal es un anticuerpo de longitud total.

20. La formulación de la reivindicación 19 en donde el anticuerpo monoclonal es un anticuerpo IgG1.

21. La formulación de la reivindicación 1 en donde el anticuerpo monoclonal es un anticuerpo humanizado.

22. La formulación de la reivindicación 1 en donde el anticuerpo monoclonal es un fragmento de anticuerpo que comprende una región de unión a antígeno.

23. La formulación de la reivindicación 22 en donde el fragmento de anticuerpo es un fragmento Fab o $F(ab')_2$.

24. La formulación de la reivindicación 1 que es estéril.

25. La formulación de la reivindicación 1 en donde el anticuerpo monoclonal une un antígeno seleccionado del grupo conformado por HER2, CD20, DR5, BR3, IgE, y VEGF.

26. La formulación de la reivindicación 25 en donde el antígeno es CD20 y el anticuerpo monoclonal es 2H7 humanizado.

27. La formulación de la reivindicación 25 en donde el antígeno es VEGF y el anticuerpo monoclonal es Bevacizumab.

28. La formulación de la reivindicación 1 en donde el anticuerpo monoclonal es susceptible a desamidación o agregación.

29. La formulación de la reivindicación 1 que es estable luego del almacenamiento a alrededor de 40°C durante por lo menos 4 semanas.

30. La formulación de la reivindicación 1 que es estable luego del almacenamiento a alrededor de 5°C o alrededor de 15°C durante por lo menos 3 meses.

31. La formulación de la reivindicación 1 que es estable luego del almacenamiento a alrededor de -20°C durante por lo menos 3 meses.

32. La formulación de la reivindicación 1 que es estable luego del congelamiento y descongelamiento.

33. La formulación de la reivindicación 1 que es acuosa.

34. La formulación de la reivindicación 1 que es congelada.

35. La formulación de la reivindicación 1 que no es liofilizada y no se ha sometido a liofilización previa.

36. La formulación de la reivindicación 35 que es acuosa y se administra a un sujeto.

37. La formulación de la reivindicación 36 en donde la formulación es para administración intravenosa (IV), subcutánea (SQ) o intramuscular (IM).

38. La formulación de la reivindicación 37 que es para administración IV y la concentración de anticuerpo es desde alrededor de 20mg/mL a alrededor de 40mg/mL.

39. La formulación de la reivindicación 37 que es para administración SQ y la concentración de anticuerpo es desde alrededor de 80mg/mL a alrededor de 250mg/mL.

315

40. Un frasquito con un tapón perforable por una jeringa, que comprende la formulación de la reivindicación 1 dentro del frasquito.

41. El frasquito de la reivindicación 40 el cual se almacena a alrededor de 2-8°C.

42. El frasquito de la reivindicación 40 el cual es un frasquito de 20cc o 50cc.

43. Un recipiente de acero inoxidable que comprende la formulación de la reivindicación 1 dentro del recipiente.

44. El recipiente de la reivindicación 43 en donde la formulación contenida en él se congela.

45. Un método de tratar una enfermedad o trastorno en un sujeto que comprende administrar la formulación de la reivindicación 1 a un sujeto en una cantidad efectiva para tratar la enfermedad o trastorno.

46. Una formulación farmacéutica que comprende:

(a) un anticuerpo IgG1 de longitud total, susceptible a desamidación o agregación en una cantidad desde alrededor de 10mg/mL a alrededor de 250mg/mL;

(b) un amortiguador de acetato de histidina, pH 5.5 a 6.5;

(c) un sacárido seleccionado del grupo conformado por trehalosa y sacarosa, en una cantidad desde alrededor de 60mM a alrededor de 250mM; y

(d) polisorbato 20 en una cantidad desde alrededor de 0.01% a alrededor de 0.1%.

47. Un método para reducir la desamidación o agregación de un anticuerpo monoclonal terapéutico, que comprende formular el anticuerpo en un amortiguador de acetato de histidina, pH 5.5 a 6.5.

48. El método de la reivindicación 47 que comprende evaluar cualquier desamidación o agregación de anticuerpo antes y después de que se formule el anticuerpo.

49. Una formulación farmacéutica que comprende un anticuerpo que se une al dominio II de HER2 en un amortiguador de histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido, y un surfactante.

50. La formulación de la reivindicación 49 en donde el amortiguador es acetato de histidina.

51. La formulación de la reivindicación 49 en donde el anticuerpo HER2 comprende las secuencias de aminoácidos pesadas variables y ligeras variables en SEQ ID Nos. 3 y 4, respectivamente.

52. La formulación de la reivindicación 51 en donde el anticuerpo HER2 comprende una secuencia de aminoácidos de cadena ligera, seleccionada de SEQ ID No. 15 y 23, y una secuencia de aminoácidos de cadena pesada, seleccionada de SEQ ID Nos. 16 y 24.

53. La formulación de la reivindicación 49 en donde el pH de la formulación es desde alrededor de 5.8 a alrededor de 6.2.

54. La formulación de la reivindicación 49 en donde el anticuerpo se une al empalme entre los dominios I, II y III de HER2.

55. La formulación de la reivindicación 49 en donde el anticuerpo es un anticuerpo de longitud total.

56. La formulación de la reivindicación 49 en donde la concentración de anticuerpo es desde alrededor de 20mg/mL a alrededor de 40mg/mL.

57. Una formulación farmacéutica que comprende Pertuzumab en una cantidad desde alrededor de 20mg/mL a alrededor de 40mg/mL, amortiguador de acetato de histidina, sacarosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5.

58. La formulación de la reivindicación 57 que comprende alrededor de 30mg/mL Pertuzumab, alrededor de 20mM de acetato de histidina, alrededor de 120mM sacarosa, y alrededor de 0.02% polisorbato 20, en donde el pH de la formulación es alrededor de 6.0.

59. Un frasquito con un tapón perforable por una jeringa, el cual comprende la formulación de la reivindicación 49.

60. Un recipiente de acero inoxidable que comprende la formulación de la reivindicación 49 en el recipiente.

61. Un método de tratar cáncer que expresa HER2 en un sujeto, que comprende administrar la formulación farmacéutica de la reivindicación 49 al sujeto en una cantidad efectiva para tratar el cáncer.

62. El método de la reivindicación 61 en donde la formulación se administra al sujeto por vía intravenosa, subcutánea, o intramuscular.

63. Un método de elaborar una formulación farmacéutica que comprende:

(a) preparar la formulación de la reivindicación 1; y

(b) evaluar la estabilidad física, la estabilidad química, o actividad biológica del anticuerpo monoclonal en la formulación.

64. Una formulación farmacéutica que comprende un anticuerpo DR5 en un amortiguador de histidina con un pH desde alrededor de 5.5 a alrededor de 6.5, un sacárido, y un surfactante.

65. La formulación de la reivindicación 64 en donde el amortiguador es acetato de histidina.

66. La formulación de la reivindicación 64 en donde el anticuerpo DR5 es un anticuerpo agonista.

67. La formulación de la reivindicación 64 en donde el anticuerpo DR5 es Apomab.

68. La formulación de la reivindicación 67 en donde el anticuerpo DR5 comprende la secuencia de aminoácidos de cadena pesada de SEQ ID No. 51, y la secuencia de aminoácidos de cadena ligera de SEQ ID No. 52.

69. La formulación de la reivindicación 64 en donde el pH de la formulación es desde alrededor de 5.8 a alrededor de 6.2.

70. La formulación de la reivindicación 64 en donde el anticuerpo es un anticuerpo de longitud total.

71. La formulación de la reivindicación 64 en donde la concentración de anticuerpo es desde alrededor de 10mg/mL a alrededor de 30mg/mL.

72. Una formulación farmacéutica que comprende Apomab en una cantidad desde alrededor de 10mg/mL a alrededor de 30mg/mL, amortiguador de acetato de histidina, trehalosa, y polisorbato 20, en donde el pH de la formulación es desde alrededor de 5.5 a alrededor de 6.5.

73. La formulación de la reivindicación 72 que comprende alrededor de 20mg/mL Apomab, alrededor de 20mM de acetato de histidina, alrededor de 240mM de trehalosa, y alrededor de 0.02% polisorbato 20, en donde el pH de la formulación es alrededor de 6.0.

74. Un frasquito con un tapón perforable por una jeringa, el cual comprende la formulación de la reivindicación 64.

75. Un recipiente de acero inoxidable que comprende la formulación de la reivindicación 64 en el recipiente.

76. Un método de tratar cáncer en un sujeto, que comprende administrar la formulación farmacéutica de la reivindicación 64 al sujeto en una cantidad efectiva para tratar cáncer.

77. El método de la reivindicación 76 en donde el cáncer es un tumor sólido.

78. El método de la reivindicación 76 en donde el cáncer es linfoma no hodgkiniano.

79. El método de la reivindicación 76 en donde la formulación se administra al sujeto por vía intravenosa, subcutánea, o intramuscular.

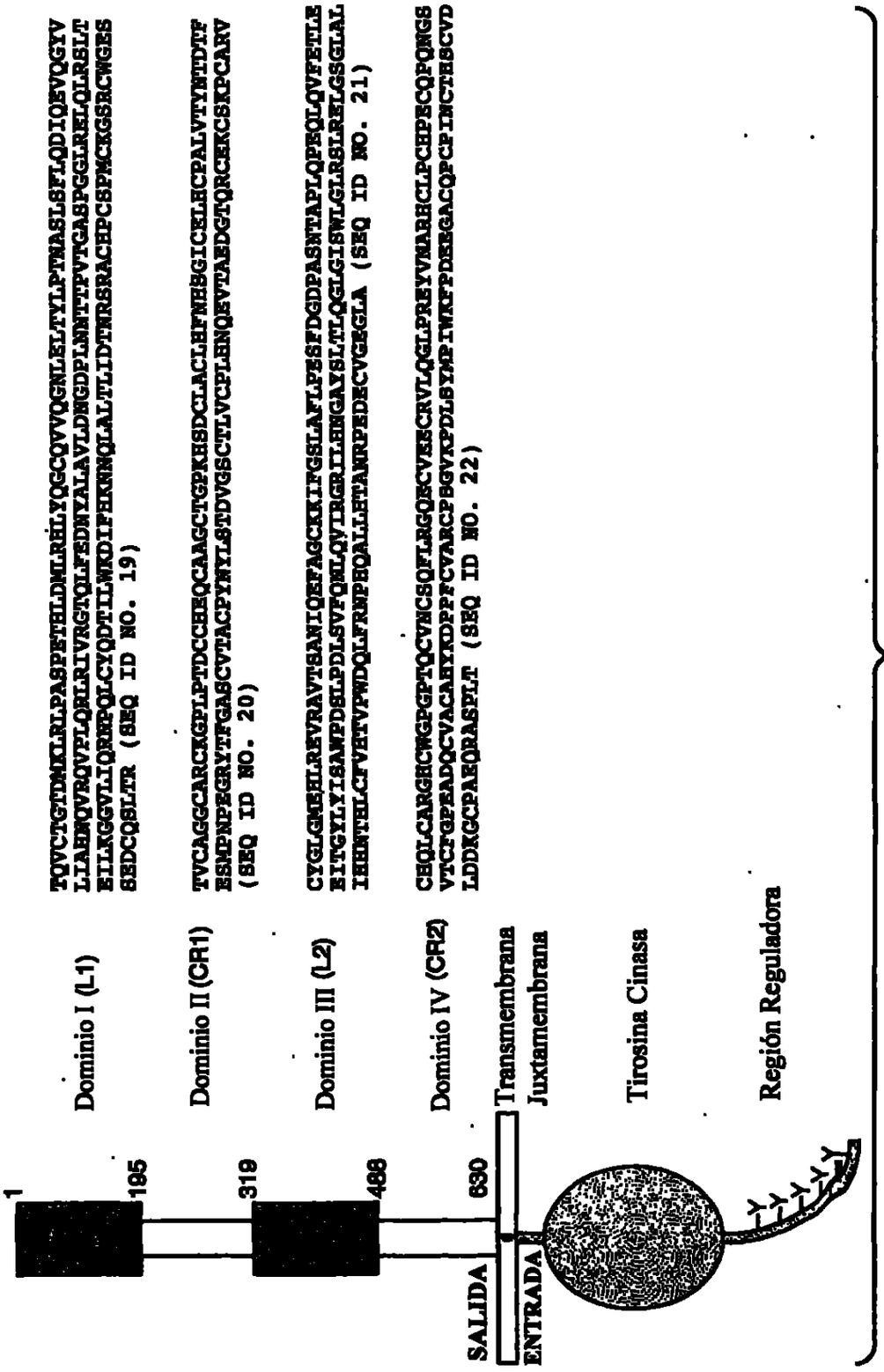

1
185
319
488
630
Dominio I (L1)
Dominio II (CR1)
Dominio III (L2)
Dominio IV (CR2)
Transmembrana
Juxtamembrana
Tirosina Cinasa
Región Reguladora
SALIDA
ENTRADA
TQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYV
LIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLT
EILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGES
SEDCQSLTR (SEQ ID NO. 19)
TVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTF
ESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARV
(SEQ ID NO. 20)
CYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLE
EITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLAL
IHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLA (SEQ ID NO. 21)
CHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQNGS
VTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVD
LDDKGCPAEQRASPLT (SEQ ID NO. 22)

FIG. 1

Variable Ligera

```
                10          20          30          40
2C4     DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
         **    **** *       *                     *
574     DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                  * *   ** ***
hum κI  DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                50          60          70          80
2C4     GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
          **                  *  *        *    * *
574     GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                   * *****
hum κI  GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                90          100
2C4     EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
           * *                 *   *
574     EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                    *** *
hum κI  EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

FIG. 2A

Variable Pesada

```
                10          20          30          40
2C4     EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
           **   **  *  * ***  *                    * *
574     EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                    ** * *
hum III EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                50 a        60          70          80
2C4     HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                    *** *    **** *
574     PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                   ****** *** ****       * * *
hum III PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

              abc       90          100ab       110
2C4     ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS (SEQ ID NO:2)
        ***  **                                **
574     QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                           ********
hum III QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

FIG. 2B

Secuencia de Aminoácidos para la Cadena Ligera de Pertuzumab

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPS

          70        80        90        100       110       120
          |         |         |         |         |         |
RFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPP

          130       140       150       160       170       180
          |         |         |         |         |         |
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

          190       200       210
          |         |         |
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

FIG. 3A

Secuencia de Aminoácidos para la Cadena Pesada de Pertuzumab

```
1         10        20        30        40        50        60
|         |         |         |         |         |         |
EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIY

          70        80        90        100       110       120
          |         |         |         |         |         |
NQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSA

          130       140       150       160       170       180
          |         |         |         |         |         |
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG

          190       200       210       220       230       240
          |         |         |         |         |         |
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP

          250       260       270       280       290       300
          |         |         |         |         |         |*
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

          310       320       330       340       350       360
          |         |         |         |         |         |
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM

          370       380       390       400       410       420
          |         |         |         |         |         |
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ

          430       440       448
          |         |         |
QGNVFSCSVMHEALHNHYTQKSLSLSPG
```

FIG. 3B

```
1                                15                                30                                45
M G W S C I I L F L V A T A T G V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S

46                               60                                75                                90
Q D V S I G V A W Y Q Q K P G K A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F

91                               105                               120                               135
T L T I S S L Q P E D F A T Y Y C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F

136                              150                               165                               180
I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E

181                              195                               210                               225
S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T

226          233
K S F N R G E C   (SEQ ID NO. 17)
```

FIG. 4A

```
1                                 15                                30                                45
M G W S C I I L F L V A T A T G V H S E V Q L V E S G G G L V Q P G G S L R L S C A A S G

46                                60                                75                                90
F T F T D Y T M D W V R Q A P G K G L E W V A D V N P N S G G S I Y N Q R F K G R F T L S

91                                105                               120                               135
V D R S K N T L Y L Q M N S L R A E D T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T

136                               150                               165                               180
V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N

181                               195                               210                               225
S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H

226                               240                               255                               270
K P S N T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D

271                               285                               300                               315
T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P R E E

316                               330                               345                               360
Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A

361                               375                               390                               405
K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N

406                               420                               435                               450
G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H

451                               465
E A L H N H Y T Q K S L S L S P G   (SEQ ID NO. 18)
```

FIG. 4B

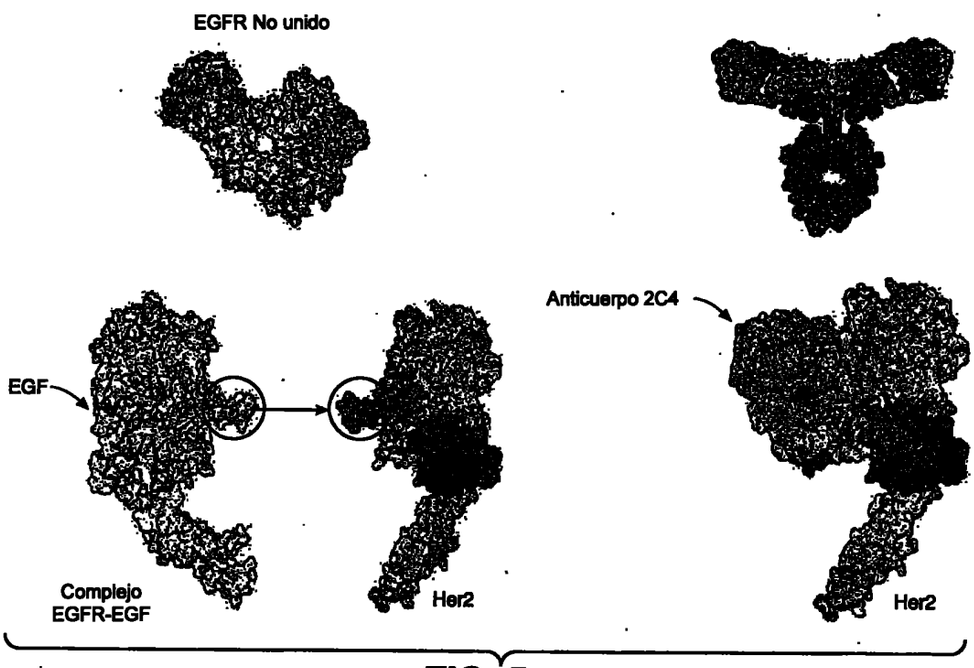

EGFR activado por ligando se heterodimeriza con HER2. 2C4 se une en el Sitio Heterodimérico de Unión
EGFR No unido
EGF
Complejo
EGFR-EGF
Her2
Anticuerpo 2C4
Her2

FIG. 5

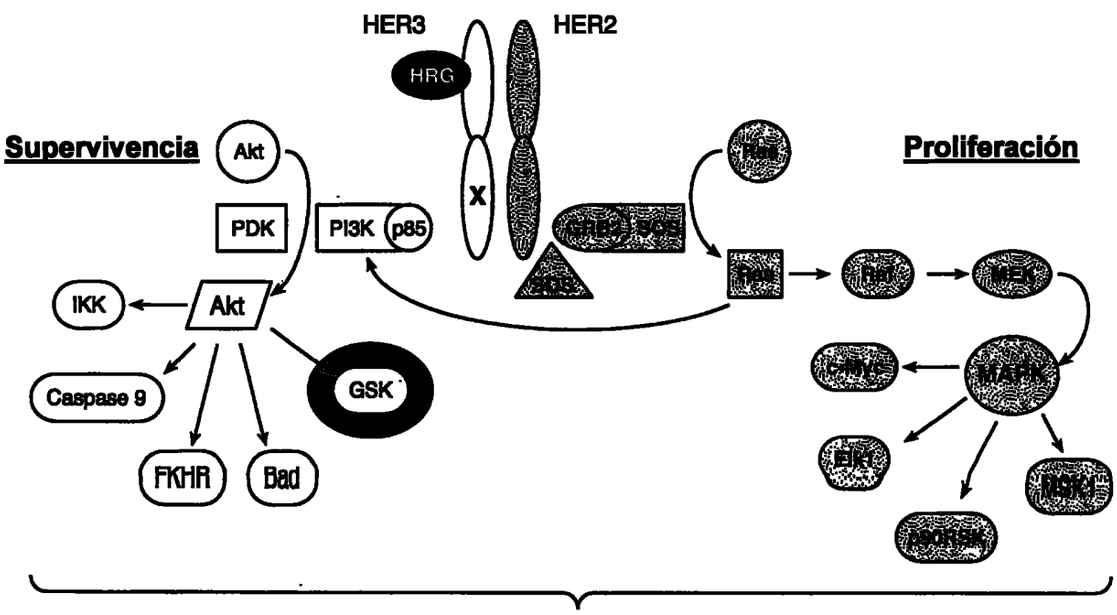

Acoplamiento de HER2/3 a las Vías MAPK y Akt
HER3
HER2
HRG
X
Supervivencia
Akt
PDK
PI3K
p85
IKK
Akt
Caspase 9
GSK
FKHR
Bad
Proliferación
Ras
Raf
MEK
c-MYC
MAPK
ERK
RSK
PKC/Src

FIG. 6

Trastuzumab
Herceptina



- Se une en IV cerca de JM.
- Protege contra el desprendimiento del receptor
- Afecta moderadamente la modulación por disminución del receptor
- Ligero efecto sobre el papel de HER2 como un co-receptor

Pertuzumab
Omnitarg



- Se une en II en la interfase de dimerización. No impide el desprendimiento del receptor
- Afecta moderadamente la modulación por disminución del receptor
- Efecto importante sobre el papel de HER2 como un co-receptor

FIG. 7

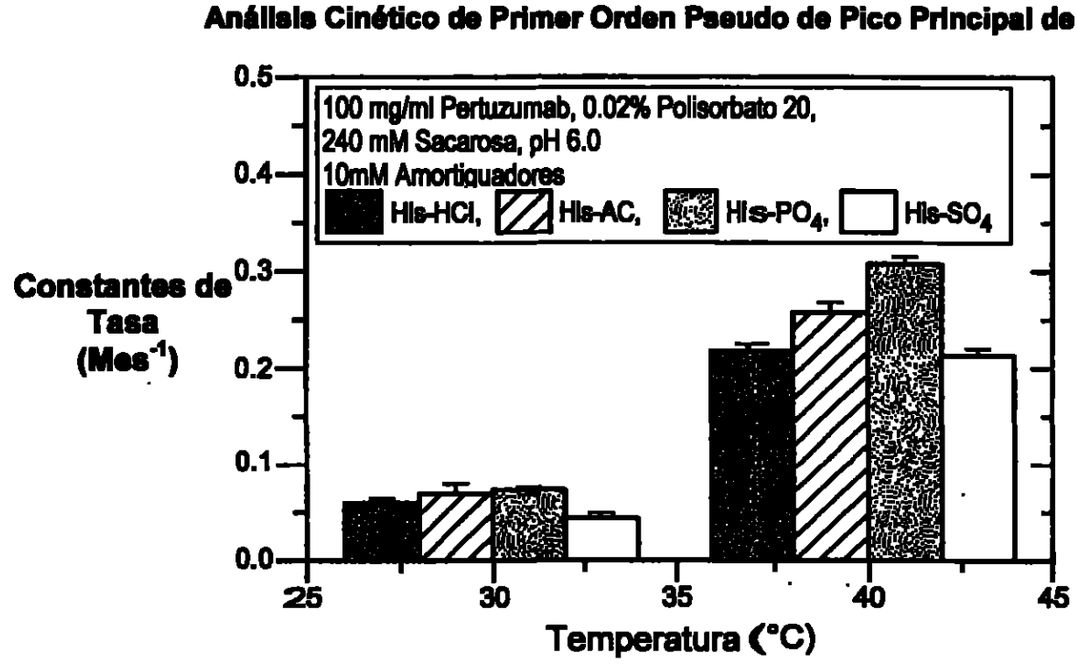

Análisis Cinético de Primer Orden Pseudo de Pico Principal de IEX
100 mg/ml Pertuzumab, 0.02% Polisorbato 20,
240 mM Sacarosa, pH 6.0
10mM Amortiguadores
His-HCl, His-AC, His-PO4, His-SO4
Constantes de Tasa (Mes-1)
0.5
0.4
0.3
0.2
0.1
0.0
25
30
35
40
45
Temperatura (°C)

FIG. 8

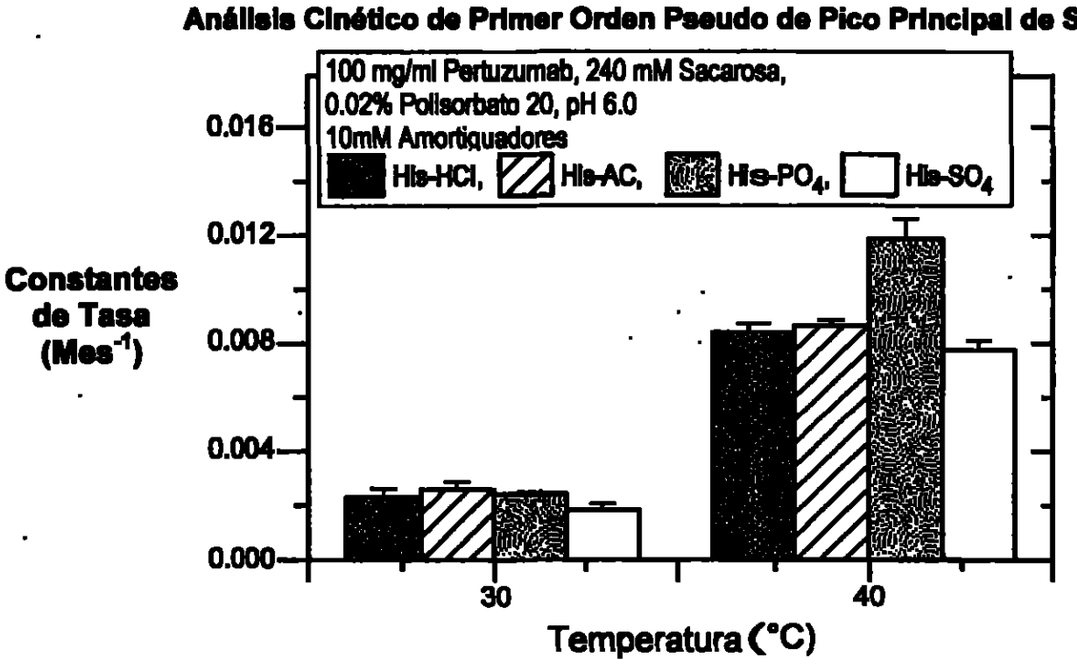

Análisis Cinético de Primer Orden Pseudo de Pico Principal de SEC
100 mg/ml Pertuzumab, 240 mM Sacarosa,
0.02% Polisorbato 20, pH 6.0
10mM Amortiguadores
His-HCl, His-AC, His-PO4, His-SO4
Constantes de Tasa (Mes-1)
0.016
0.012
0.008
0.004
0.000
30
40
Temperatura (°C)

FIG. 9

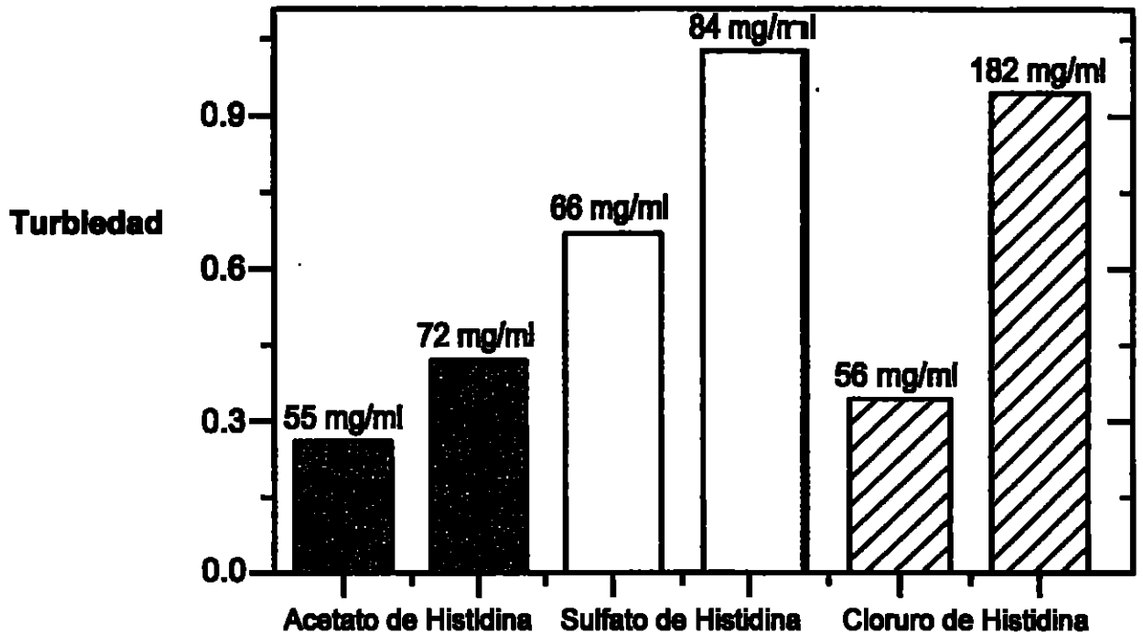

Turbiedad
0.0
0.3
0.6
0.9
55 mg/ml
72 mg/ml
66 mg/ml
84 mg/ml
56 mg/ml
182 mg/ml
Acetato de Histidina
Sulfato de Histidina
Cloruro de Histidina

FIG. 10

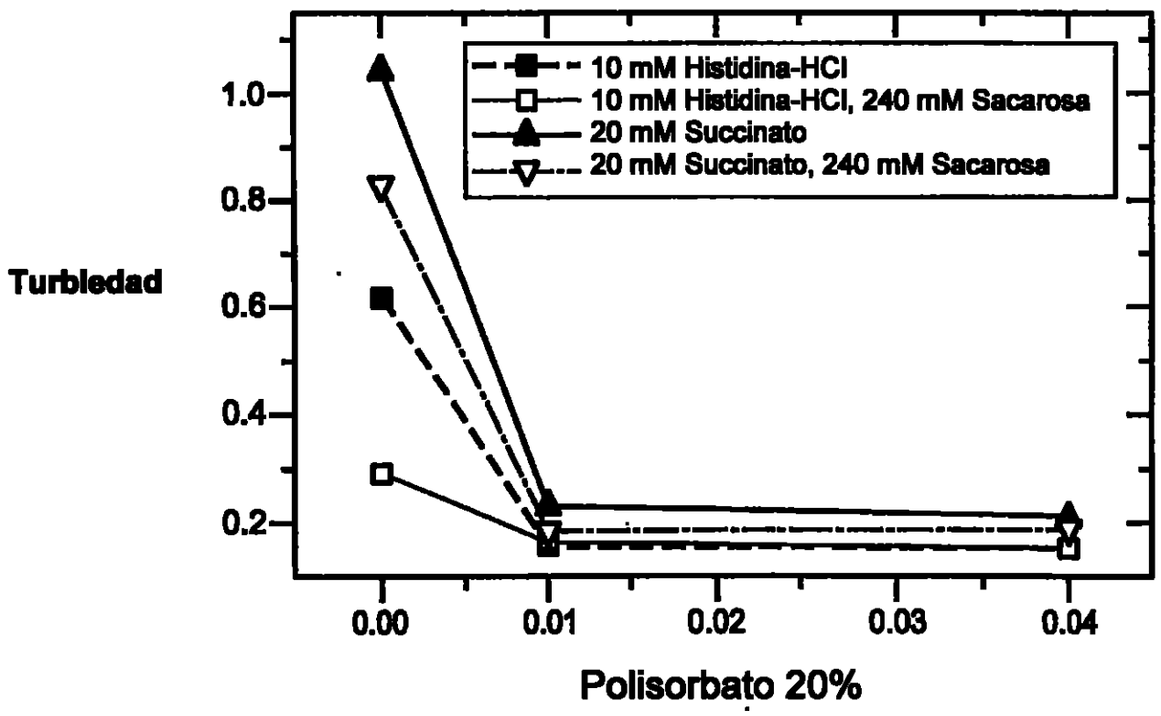

Turbiedad
0.2
0.4
0.6
0.8
1.0
10 mM Histidina-HCl
10 mM Histidina-HCl, 240 mM Sacarosa
20 mM Succinato
20 mM Succinato, 240 mM Sacarosa
0.00
0.01
0.02
0.03
0.04
Polisorbato 20%

FIG. 11

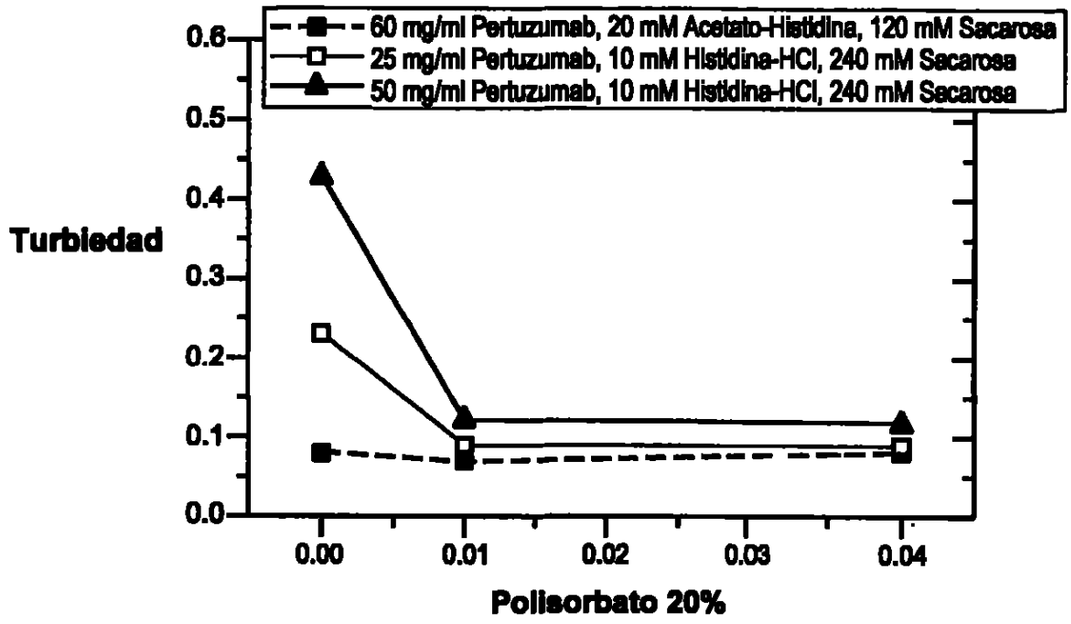

60 mg/ml Pertuzumab, 20 mM Acetato-Histidina, 120 mM Sacarosa
25 mg/ml Pertuzumab, 10 mM Histidina-HCl, 240 mM Sacarosa
50 mg/ml Pertuzumab, 10 mM Histidina-HCl, 240 mM Sacarosa
Turbiedad
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.01
0.02
0.03
0.04
Polisorbato 20%

FIG. 12

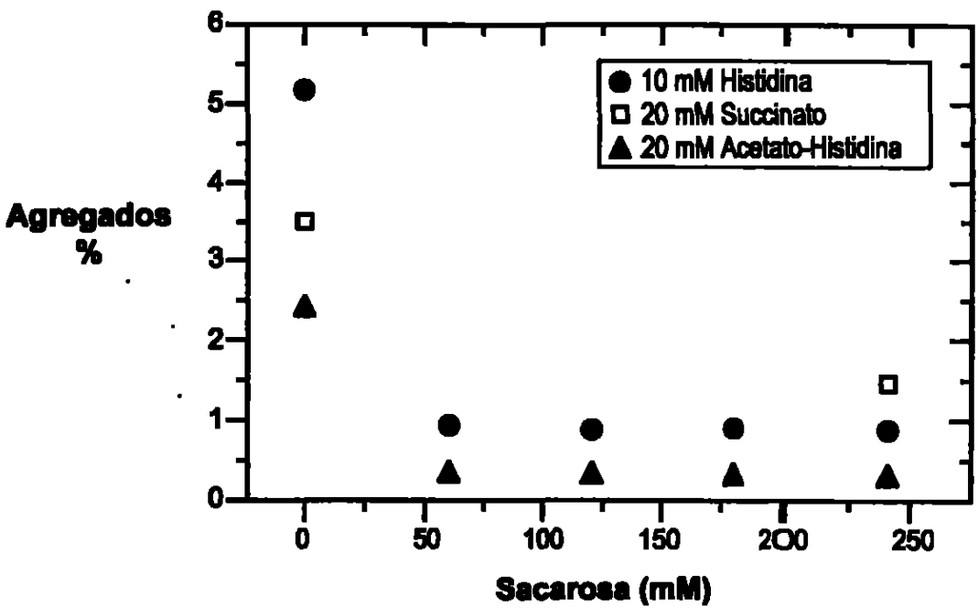

10 mM Histidina
20 mM Succinato
20 mM Acetato-Histidina
Agregados %
6
5
4
3
2
1
0
0
50
100
150
200
250
Sacarosa (mM)

FIG. 13

Cadena Ligera

```
1                   15                  30                  45
D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V N T A V A W Y Q Q K P G K A P K

46                  60                  75                  90
L L I Y S A S F L Y S G V P S R F S G S R S G T D F T L T I S S L Q P E D F A T Y Y C Q Q

91                  105                 120                 135
H Y T T P P T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L

136                 150                 165                 180
L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T

181                 195                 210 214
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C
```

FIG. 14A

Cadena Pesada

```
1                                                                  45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F N I K D T Y I H W V R Q A P G K G L

46                                                                 90
E W V A R I Y P T N G Y T R Y A D S V K G R F T I S A D T S K N T A Y L Q M N S L R A E D

91                                                                135
T A V Y Y C S R W G G D G F Y A M D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S

136                                                               180
K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

181                                                               225
G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K

226                                                               270
T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S

271                                                               315
H E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D

316                                                               360
W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E

361                                                               405
M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G

406                                                          449
S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G
```

FIG. 14B

```
1                                15                                30                                45
V H S D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q D V S I G V A W Y Q Q K P G K

46                               60                                75                                90
A P K L L I Y S A S Y R Y T G V P S R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y

91                               105                               120                               135
C Q Q Y Y I Y P Y T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V

136                              150                               165                               180
V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S

181                              195                               210          217
T L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C   (SEQ ID NO. 23)
```

FIG. 15A

```
1                                15                                30                                45
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T D Y T M D W V R Q A P G K G L

46                               60                                75                                90
E W V A D V N P N S G G S I Y N Q R F K G R F T L S V D R S K N T L Y L Q M N S L R A E D

91                               105                               120                               135
T A V Y Y C A R N L G P S F Y F D Y W G Q G T L V T V S S A S T K G P S V F P L A P S S K

136                              150                               165                               180
S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G

181                              195                               210                               225
L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K T

226                              240                               255                               270
H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H

271                              285                               300                               315
E D P E V K F N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W

316                              330                               345                               360
L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M

361                              375                               390                               405
T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S

406                              420                               435                               449
F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
```

(SEQ ID NO. 24)

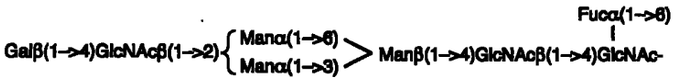
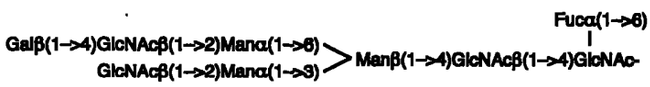
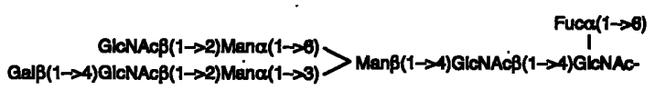
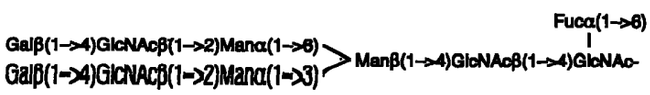
FIG. 15B

FIG. 16A

Estructuras de Oligosacáridos Observadas Comúnmente en Anticuerpos IgG

Estructuras	Abreviatura	Mass
Manα(1–>6) Manα(1–>3) > Manα(1–>6) Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	Man5	1235
GlcNAcβ(1–>2){ Manα(1–>6) Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc- (Fucα(1–>6) en GlcNAc)	G-1	1260
GlcNAcβ(1–>2)Manα(1–>6) GlcNAcβ(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	G0-F	1317
Manα(1–>6) Manα(1–>3) > Manα(1–>6) Manα(1–>2)Manα(1–>3) > Manβ(1–>4)GlcNAcβ(1–>4)GlcNAc-	Man6	1398

Estructuras de Oligosacáridos Observadas Comúnmente en Anticuerpos IgG

Structures	Abreviatura	Masa
	G1-1	1423
	G0	1463
	G1 (1-6)	1626
	G1 (1-3)	1626
	G2	1788

Las masas mostradas en esta Figura corresponden a los valores $(M+Na)^+$

FIG. 16B

Anticuerpos Anti-IgE: Cadena Ligera (Dominios V_L y C_L)

```
                10         20         30         40         50         60         70         80
E25     DIQLTQSPSS LSASVGDRVT ITC[RASQSVD YDGDSYMN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
E26     DIQLTQSPSS LSASVGDRVT ITC[RASKSVD GSGDSYLN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
HAE1    DIQLTQSPSS LSASVGDRVT ITC[RASKSVD GSGDSYLN]WY QQKPGKAPKL LIY[AASYLES] GVPSRFSGSG SGTDFTLTIS
Hu-901  DI□LTQSP□T LS□S□G□RAT LSC RASQS□G TNIH---- WY QQKPG□APRL LI□ YASES□S G□PSRFSGSG SGTDFTLTIS

              90         100        110     C_L starts
E25     SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
E26     SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
HAE1    SLQPEDFATY YC[QQSHEDPY T]FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
Hu-901  □L□PEDFA□Y YC QQSDSW□T T FGQGTKVEI KRT VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD

E25     SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 37
E26     SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 38
HAE1    SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 39
Hu-901  SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO.: 40
```

FIG. 17A

Anticuerpos Anti-IgE: Cadena Pesada (Dominios V_H y C_H)

```
                10        20        30        40        50 a      60        70        80        90
E25     EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWHW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
E26     EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWHW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
HAE1    EVQLVESGGG LVQPGGSLRL SCAVSGYSIT S[GYSWHW]IRQ APGKGLEWVA [SITYDGSTNY NPSVKG]RITI SRDDSKNTFY LQMNSLRAED
Hu-901  QVQLVQSGAE VKKPGASVKV SCKASGYTF- S SYWLH WVRQ APGQGLEWMG EISPGYTSTN YNEKFKS KATL TADKSTSTAY MELSSLRSED

                100       110ab       120 C_H starts
E25     TAVYYCAR[GS HYFGHWHFAV] WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
E26     TAVYYCAR[GS HYFGHWHFAV] WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
HAE1    TAVYYCAR[GS HYFGHWHFAV] WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
Hu-901  TAVYYCAR GS HFGSWYDYFDY WGQGTLVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS

E25       VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
E26       VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
HAE1      VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE
Hu-901    VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

E25       VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
E26       VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
HAE1      VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
Hu-901    VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE

E25       SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 41
E26       SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 42
HAE1      SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 43
Hu-901    SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO.: 44
```

FIG. 17B

Alineación de Secuencias de Dominios Ligeros Variables

```
                    |----------FR1----------|      CDR1      |----
                              10        20          30          40
2H7                 QIVLSQSPAILSASPGEKVTMTC [RASSSVS-YMH] WYQQKP
                    * ***    **     * **
hu2H7.v16           DIQMTQSPSSLSASVGDRVTITC [RASSSVS-YMH] WYQQKP
                                            * * * **
hum kI              DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                    --FR2--|    CDR2    |----------FR3-----
                          50          60        70        80
2H7                 GSSPKPWIY [APSNLAS] GVPARFSGSGSGTSYSLTISRVEA
                     **   *                *         ***   ****
hu2H7.v16           GKAPKPLIY [APSNLAS] GVPSRFSGSGSGTDFTLTISSLQP
                       *      * * *
hum kI              GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                    --------|    CDR3    |--FR4---|
                          90          100
2H7                 EDAATYYC [QQWSFNPPT] FGAGTKLELKR
                      *                  *   * *
hu2H7.v16           EDFATYYC [QQWSFNPPT] FGQGTKVEIKR
                             * **** *
hum kI              EDFATYYC [QQYNSLPWT] FGQGTKVEIKR
```

FIG. 18A

Alineación de Secuencias de Dominios Pesados Variables

```
                    |----------FR1----------|      CDR1      |----
                              10        20          30          40
2H7                 QAYLQQSGAELVRPGASVKMSCKAS [GYTFTSYNMH] WVKQT
                    *** **  **  *  * ***  *                  * *
hu2H7.v16           EVQLVESGGGLVQPGGSLRLSCAAS [GYTFTSYNMH] WVRQA
                                              * *  * *
hum III             EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                    --FR2--|        CDR2        |-------FR3-------
                          50  a       60          70          80
2H7                 PRQGLEWIG [AIYPGNGDTSYNQKFKG] KATLTVDKSSSTAYM
                     **   *                      ** **    ** * *
hu2H7.v16           PGKGLEWVG [AIYPGNGDTSYNQKFKG] RFTISVDKSKNTLYL
                            *  * **** * * ****      * *
hum III             PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

                    ----------------|      CDR3      |---FR4---|
                      abc       90          100abcde        110
2H7                 QLSSLTSEDSAVYFCAR [VVYYSNSYWYFDV] WGTGTTVTVSS
                     **  **  *   *                      *
hu2H7.v16           QMNSLRAEDTAVYYCAR [VVYYSNSYWYFDV] WGQGTLVTVSS
                                      ******* *** *
hum III             QMNSLRAEDTAVYYCAR [GRVGYSLY---DY] WGQGTLVTVSS
```

FIG. 18B

Secuencias de Dominio Variable de Anticuerpo Anti-VEGF

Secuencia ligera variable anti-VEGF:

DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKPDGTVKVLIYFTSSLHSGVPSRFSGS
GSGTDYSLTISNLEPEDIATYYCQQYSTVPWTFGGGTKLEIK
(SEQ ID NO: 31)

Secuencia pesada variable anti-VEGF:

EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGEPTYAADFK
RRFTFSLETSASTAYLQISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGAGTTVTVSS
(SEQ ID NO: 32)

Secuencia ligera variable del anticuerpo anti-VEGF:

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK
(SEQ ID NO: 33)

Secuencia pesada variable de anticuerpo anti-VEGF:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFK
RRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS
(SEQ ID NO: 34)

Secuencia ligera variable de anticuerpo anti-VEGF:

DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK
(SEQ ID NO: 35)

Secuencia pesada variable de anticuerpo anti-VEGF:

EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFK
RRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSS
(SEQ ID NO: 36)

FIG. 19

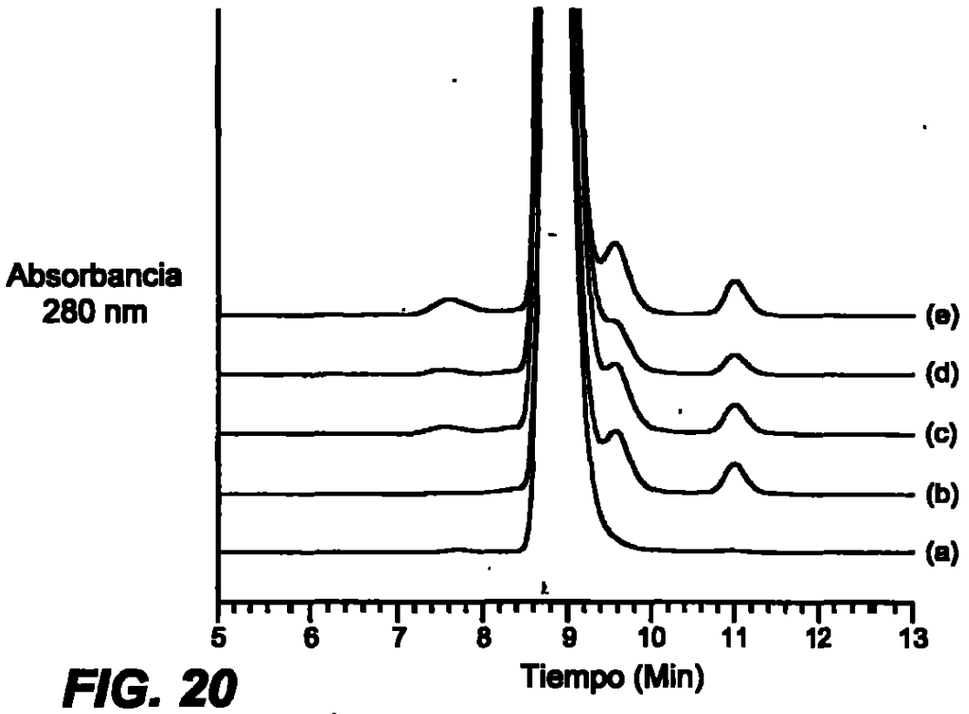

Absorbancia
280 nm
(e)
(d)
(c)
(b)
(a)
5
6
7
8
9
10
11
12
13
Tiempo (Min)

FIG. 20

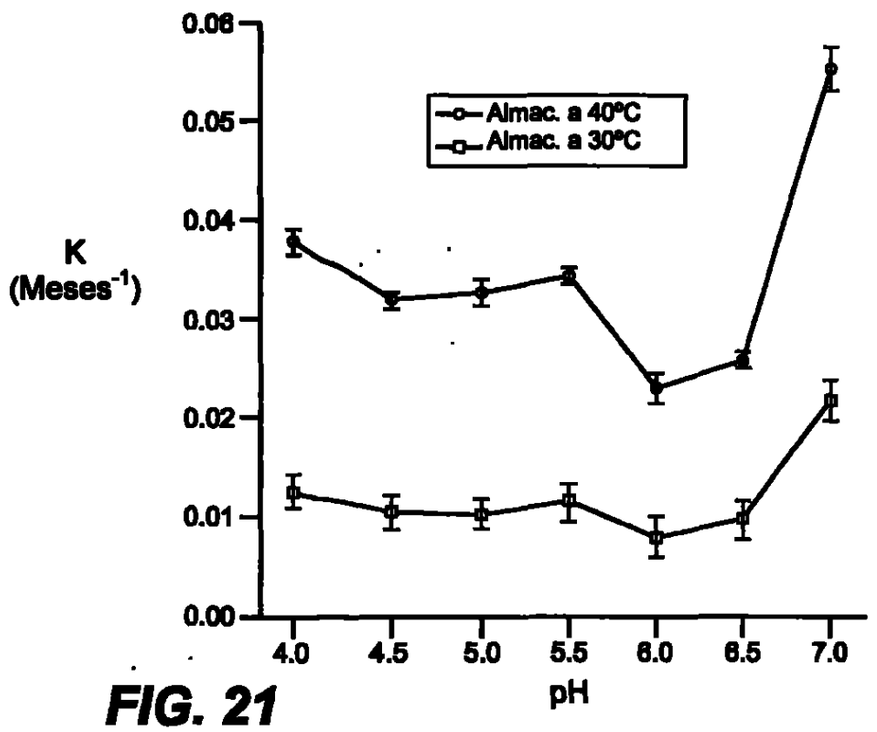

0.06
0.05
0.04
0.03
0.02
0.01
0.00
K
(Meses^{-1})
Almac. a 40°C
Almac. a 30°C
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH

FIG. 21

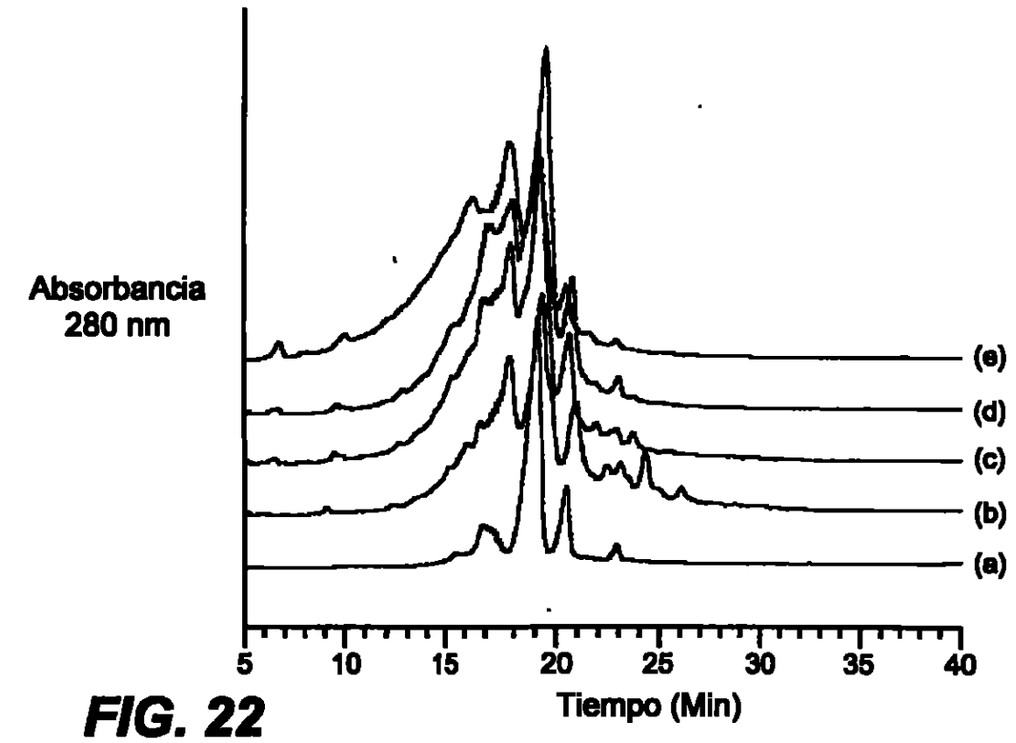

Absorbancia
280 nm
(e)
(d)
(c)
(b)
(a)
5
10
15
20
25
30
35
40
Tiempo (Min)

FIG. 22

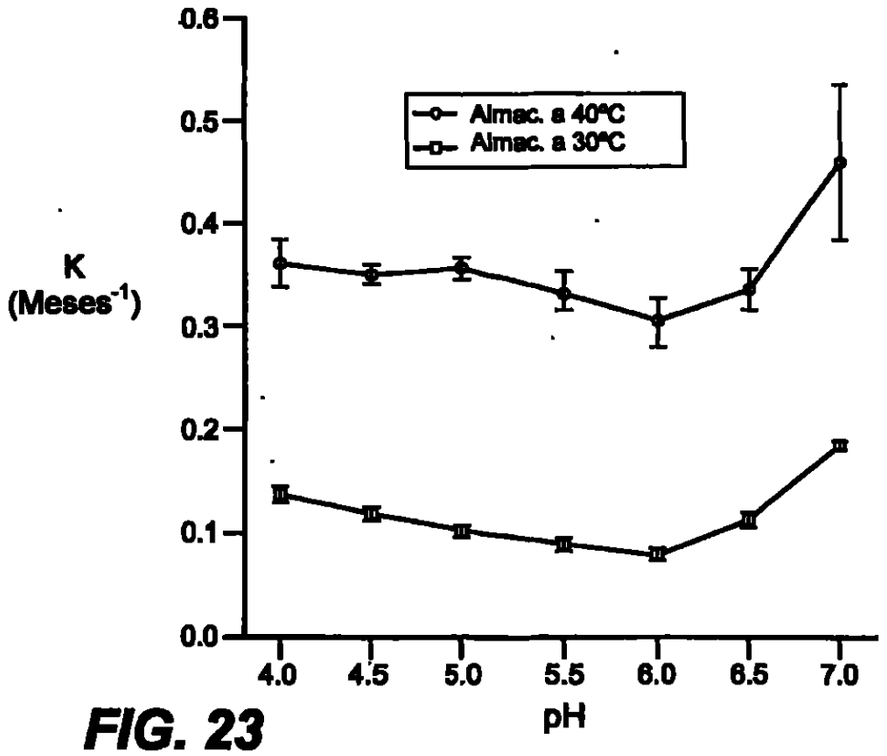

Almac. a 40°C
Almac. a 30°C
K
(Meses^{-1})
0.6
0.5
0.4
0.3
0.2
0.1
0.0
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH

FIG. 23

```
   1 TTTCCTCACTGACTATAAAAGAATAGAGAAGGAAGGGCTTCAGTGACCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC

   1 ATGGCTATGATGGAGGTCCAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGT
   1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlyGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys

 181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAA
  31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu

 271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG
  61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys

 361 ATGATTTTGAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCNCAG
  91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln

 451 AGAGTAGCAGCTCACATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAA
 121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys

 541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGG
 151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly

 631 TTTTACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATT
 181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle

 721 TACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT
 211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr

 811 TCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCAT
 241 SerIleTyrGlnGlyGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis

 901 GAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGAT
 271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp

 991 GATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAA
```

FIG. 24

```
   1 CCCACGCGTC CGCATAAATC AGCACGCGGC CGGAGAACCC CGCAATCTCT GCGCCCACAA AATACACCGA CGATGCCCGA TCTACTTTAA GGGCTGAAAC
     GGGTGCGCAG GCGTATTTAG TCGTGCGCCG GCCTCTTGGG GCGTTAGAGA CGCGGGTGTT TTATGTGGCT GCTACGGGCT AGATGAAATT CCCGACTTTG
 101 CCACGGGCCT GAGAGACTAT AAGAGCGTTC CCTACCGCCA TGGAACAACG GGGACAGAAC GCCCCGGCCG CTTCGGGGGC CCGGAAAAGG CACGGCCCAG
     GGTGCCCGGA CTCTCTGATA TTCTCGCAAG GGATGGCGGT ACCTTGTTGC CCCTGTCTTG CGGGGCCGGC GAAGCCCCCG GGCCTTTTCC GTGCCGGGTC
   1                                 M etGluGlnAr gGlyGlnAsn AlaProAlaA laSerGlyAl aArgLysArg HisGlyProGly
 201 GACCCAGGGA GGCGCGGGGA GCCAGGCCTG GGCTCCGGGT CCCCAAGACC CTTGTGCTCG TTGTCGCCGC GGTCCTGCTG TTGGTCTCAG CTGAGTCTGC
     CTGGGTCCCT CCGCGCCCCT CGGTCCGGAC CCGAGGCCCA GGGGTTCTGG GAACACGAGC AACAGCGGCG CCAGGACGAC AACCAGAGTC GACTCAGACG
  22   ProArgGl uAlaArgGly AlaArgProG lyLeuArgVa lProLysThr LeuValLeuV alValAlaAl aValLeuLeu LeuValSerA laGluSerAla
 301 TCTGATCACC CAACAAGACC TAGCTCCCCA GCAGAGAGCG GCCCCACAAC AAAAGAGGTC CAGCCCCTCA GAGGGATTGT GTCCACCTGG ACACCATATC
     AGACTAGTGG GTTGTTCTGG ATCGAGGGGT CGTCTCTCGC CGGGGTGTTG TTTTCTCCAG GTCGGGGAGT CTCCCTAACA CAGGTGGACC TGTGGTATAG
  55   LeuIleThr GlnGlnAspL euAlaProGl nGlnArgAla AlaProGlnG lnLysArgSe rSerProSer GluGlyLeuC ysProProGl yHisHisIle
 401 TCAGAAGACG GTAGAGATTG CATCTCCTGC AAATATGGAC AGGACTATAG CACTCACTGG AATGACCTCC TTTTCTGCTT GCGCTGCACC AGGTGTGATT
     AGTCTTCTGC CATCTCTAAC GTAGAGGACG TTTATACCTG TCCTGATATC GTGAGTGACC TTACTGGAGG AAAAGACGAA CGCGACGTGG TCCACACTAA
  88 SerGluAspG lyArgAspCy sIleSerCys LysTyrGlyG lnAspTyrSe rThrHisTrp AsnAspLeuL euPheCysLe uArgCysThr ArgCysAspSer
 501 CAGGTGAAGT GGAGCTAAGT CCCTGCACCA CGACCAGAAA CACAGTGTGT CAGTGCGAAG AAGGCACCTT CCGGGAAGAA GATTCTCCTG AGATGTGCCG
     GTCCACTTCA CCTCGATTCA GGGACGTGGT GCTGGTCTTT GTGTCACACA GTCACGCTTC TTCCGTGGAA GGCCCTTCTT CTAAGAGGAC TCTACACGGC
 122   GlyGluVa lGluLeuSer ProCysThrT hrThrArgAs nThrValCys GlnCysGluG luGlyThrPh eArgGluGlu AspSerProG luMetCysArg
 601 GAAGTGCCGC ACAGGGTGTC CCAGAGGGAT GGTCAAGGTC GGTGATTGTA CACCCTGGAG TGACATCGAA TGTGTCCACA AAGAATCAGG CATCATCATA
     CTTCACGGCG TGTCCCACAG GGTCTCCCTA CCAGTTCCAG CCACTAACAT GTGGGACCTC ACTGTAGCTT ACACAGGTGT TTCTTAGTCC GTAGTAGTAT
 155   LysCysArg ThrGlyCysP roArgGlyMe tValLysVal GlyAspCysT hrProTrpSe rAspIleGlu CysValHisL ysGluSerGl yIleIleIle
 701 GGAGTCACAG TTGCAGCCGT AGTCTTGATT GTGGCTGTGT TTGTTTGCAA GTCTTTACTG TGGAAGAAAG TCCTTCCTTA CCTGAAAGGC ATCTGCTCAG
     CCTCAGTGTC AACGTCGGCA TCAGAACTAA CACCGACACA AACAAACGTT CAGAAATGAC ACCTTCTTTC AGGAAGGAAT GGACTTTCCG TAGACGAGTC
 188 GlyValThrV alAlaAlaVa lValLeuIle ValAlaValP heValCysLy sSerLeuLeu TrpLysLysV alLeuProTy rLeuLysGly IleCysSerGly
 801 GTGGTGGTGG GGACCCTGAG CGTGTGGACA GAAGCTCACA ACGACCTGGG GCTGAGGACA ATGTCCTCAA TGAGATCGTG AGTATCTTGC AGCCCACCCA
     CACCACCACC CCTGGGACTC GCACACCTGT CTTCGAGTGT TGCTGGACCC CGACTCCTGT TACAGGAGTT ACTCTAGCAC TCATAGAACG TCGGGTGGGT
 222   GlyGlyGl yAspProGlu ArgValAspA rgSerSerGl nArgProGly AlaGluAspA snValLeuAs nGluIleVal SerIleLeuG lnProThrGln
```

FIG. 25A

```
 901 GGTCCCTGAG CAGGAAATGG AAGTCCAGGA GCCAGCAGAG CCAACAGGTG TCAACATGTT GTCCCCCGGG GAGTCAGAGC ATCTGCTGGA ACCGGCAGAA
     CCAGGGACTC GTCCTTTACC TTCAGGTCCT CGGTCGTCTC GGTTGTCCAC AGTTGTACAA CAGGGGGCCC CTCAGTCTCG TAGACGACCT TGGCCGTCTT
 255  ValProGlu GlnGluMetG luValGlnGl uProAlaGlu ProThrGlyV alAsnMetLe uSerProGly GluSerGluH isLeuLeuGl uProAlaGlu

1001 GCTGAAAGGT CTCAGAGGAG GAGGCTGCTG GTTCCAGCAA ATGAAGGTGA TCCCACTGAG ACTCTGAGAC AGTGCTTCGA TGACTTTGCA GACTTGGTGC
     CGACTTTCCA GAGTCTCCTC CTCCGACGAC CAAGGTCGTT TACTTCCACT AGGGTGACTC TGAGACTCTG TCACGAAGCT ACTGAAACGT CTGAACCACG
 288 AlaGluArgS erGlnArgAr gArgLeuLeu ValProAlaA snGluGlyAs pProThrGlu ThrLeuArgG lnCysPheAs pAspPheAla AspLeuValPro

1101 CCTTTGACTC CTGGGAGCCG CTCATGAGGA AGTTGGGCCT CATGGACAAT GAGATAAAGG TGGCTAAAGC TGAGGCAGCG GGCCACAGGG ACACCTTGTA
     GGAAACTGAG GACCCTCGGC GAGTACTCCT TCAACCCGGA GTACCTGTTA CTCTATTTCC ACCGATTTCG ACTCCGTCGC CCGGTGTCCC TGTGGAACAT
 322   PheAspSe rTrpGluPro, LeuMetArgL ysLeuGlyLe uMetAspAsn GluIleLysV alAlaLysAl aGluAlaAla GlyHisArgA spThrLeuTyr

1201 CACGATGCTG ATAAAGTGGG TCAACAAAAC CGGGCGAGAT GCCTCTGTCC ACACCCTGCT GGATGCCTTG GAGACGCTGG GAGAGAGACT TGCCAAGCAG
     GTGCTACGAC TATTTCACCC AGTTGTTTTG GCCCGCTCTA CGGAGACAGG TGTGGGACGA CCTACGGAAC CTCTGCGACC CTCTCTCTGA ACGGTTCGTC
 355  ThrMetLeu IleLysTrpV alAsnLysTh rGlyArgAsp AlaSerValH isThrLeuLe uAspAlaLeu GluThrLeuG lyGluArgLe uAlaLysGln

1301 AAGATTGAGG ACCACTTGTT GAGCTCTGGA AAGTTCATGT ATCTAGAAGG TAATGCAGAC TCTGCCWTGT CCTAAGTGTG ATTCTCTTCA GGAAGTGAGA
     TTCTAACTCC TGGTGAACAA CTCGAGACCT TTCAAGTACA TAGATCTTCC ATTACGTCTG AGACGGAACA GGATTCACAC TAAGAGAAGT CCTTCACTCT
 388 LysIleGluA spHisLeuLe uSerSerGly LysPheMetT yrLeuGluGl yAsnAlaAsp SerAlaXaaS erOC*

1401 CCTTCCCTGG TTTACCTTTT TTCTGGAAAA AGCCCAACTG GACTCCAGTC AGTAGGAAAG TGCCACAATT GTCACATGAC CGGTACTGGA AGAAACTCTC
     GGAAGGGACC AAATGGAAAA AAGACCTTTT TCGGGTTGAC CTGAGGTCAG TCATCCTTTC ACGGTGTTAA CAGTGTACTG GCCATGACCT TCTTTGAGAG

1501 CCATCCAACA TCACCCAGTG GATGGAACAT CCTGTAACTT TTCACTGCAC TTGGCATTAT TTTTATAAGC TGAATGTGAT AATAAGGACA CTATGGAAAT
     GGTAGGTTGT AGTGGGTCAC CTACCTTGTA GGACATTGAA AAGTGACGTG AACCGTAATA AAAATATTCG ACTTACACTA TTATTCCTGT GATACCTTTA

1601 GTCTGGATCA TTCCGTTTGT GCGTACTTTG AGATTTGGTT TGGGATGTCA TTGTTTTCAC AGCACTTTTT TATCCTAATG TAAATGCTTT ATTTATTTAT
     CAGACCTAGT AAGGCAAACA CGCATGAAAC TCTAAACCAA ACCCTACAGT AACAAAAGTG TCGTGAAAAA ATAGGATTAC ATTTACGAAA TAAATAAATA

1701 TTGGGCTACA TTGTAAGATC CATCTACAAA AAAAAAAAAA AAAAAAAAAG GGCGGCCGCG ACTCTAGAGT CGACCTGCAG AAGCTTGGCC GCCATGGCC
     AACCCGATGT AACATTCTAG GTAGATGTTT TTTTTTTTTT TTTTTTTTTC CCGCCGGCGC TGAGATCTCA GCTGGACGTC TTCGAACCGG CGGTACCGG
```

FIG. 25B

```
ATG GAA CAA CGG GGA CAG AAC GCC CCG GCC GCT TCG GGG GCC CGG AAA     48
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg Lys
  1               5                  10                  15

AGG CAC GGC CCA GGA CCC AGG GAG GCG CGG GGA GCC AGG CCT GGG CCC     96
Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro Gly Pro
            20                  25                  30

CGG GTC CCC AAG ACC CTT GTG CTC GTT GTC GCC GCG GTC CTG CTG TTG    144
Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val Leu Leu Leu
        35                  40                  45

GTC TCA GCT GAG TCT GCT CTG ATC ACC CAA CAA GAC CTA GCT CCC CAG    192
Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp Leu Ala Pro Gln
    50                  55                  60

CAG AGA GCG GCC CCA CAA CAA AAG AGG TCC AGC CCC TCA GAG GGA TTG    240
Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser Pro Ser Glu Gly Leu
65                  70                  75                  80

TGT CCA CCT GGA CAC CAT ATC TCA GAA GAC GGT AGA GAT TGC ATC TCC    288
Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
                85                  90                  95

TGC AAA TAT GGA CAG GAC TAT AGC ACT CAC TGG AAT GAC CTC CTT TTC    336
Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu Leu Phe
            100                 105                 110

TGC TTG CGC TGC ACC AGG TGT GAT TCA GGT GAA GTG GAG CTA AGT CCG    384
Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
        115                 120                 125

TGC ACC ACG ACC AGA AAC ACA GTG TGT CAG TGC GAA GAA GGC ACC TTC    432
Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140

CGG GAA GAA GAT TCT CCT GAG ATG TGC CGG AAG TGC CGC ACA GGG TGT    480
Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
145                 150                 155                 160

CCC AGA GGG ATG GTC AAG GTC GGT GAT TGT ACA CCC TGG AGT GAC ATC    528
Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175

GAA TGT GTC CAC AAA GAA TCA GGT ACA AAG CAC AGT GGG GAA GCC CCA    576
Glu Cys Val His Lys Glu Ser Gly Thr Lys His Ser Gly Glu Ala Pro
            180                 185                 190

GCT GTG GAG GAG ACG GTG ACC TCC AGC CCA GGG ACT CCT GCC TCT CCC    624
Ala Val Glu Glu Thr Val Thr Ser Ser Pro Gly Thr Pro Ala Ser Pro
        195                 200                 205

TGT TCT CTC TCA GGC ATC ATC ATA GGA GTC ACA GTT GCA GCC GTA GTC    672
Cys Ser Leu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
    210                 215                 220
```

FIG. 26A

```
TTG ATT GTG GCT GTG TTT GTT TGC AAG TCT TTA CTG TGG AAG AAA GTC   720
Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys Val
225                 230                 235                 240

CTT CCT TAC CTG AAA GGC ATC TGC TCA GGT GGT GGT GGG GAC CCT GAG   768
Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp Pro Glu
                245                 250                 255

CGT GTG GAC AGA AGC TCA CAA CGA CCT GGG GCT GAG GAC AAT GTC CTC   816
Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp Asn Val Leu
            260                 265                 270

AAT GAG ATC GTG AGT ATC TTG CAG CCC ACC CAG GTC CCT GAG CAG GAA   864
Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro Glu Gln Glu
        275                 280                 285

ATG GAA GTC CAG GAG CCA GCA GAG CCA ACA GGT GTC AAC ATG TTG TCC   912
Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn Met Leu Ser
    290                 295                 300

CCC GGG GAG TCA GAG CAT CTG CTG GAA CCG GCA GAA GCT GAA AGG TCT   960
Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala Glu Arg Ser
305                 310                 315                 320

CAG AGG AGG AGG CTG CTG GTT CCA GCA AAT GAA GGT GAT CCC ACT GAG  1008
Gln Arg Arg Arg Leu Leu Val Pro Ala Asn Glu Gly Asp Pro Thr Glu
                325                 330                 335

ACT CTG AGA CAG TGC TTC GAT GAC TTT GCA GAC TTG GTG CCC TTT GAC  1056
Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val Pro Phe Asp
            340                 345                 350

TCC TGG GAG CCG CTC ATG AGG AAG TTG GGC CTC ATG GAC AAT GAG ATA  1104
Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp Asn Glu Ile
        355                 360                 365

AAG GTG GCT AAA GCT GAG GCA GCG GGC CAC AGG GAC ACC TTG TAC ACG  1152
Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr Leu Tyr Thr
    370                 375                 380

ATG CTG ATA AAG TGG GTC AAC AAA ACC GGG CGA GAT GCC TCT GTC CAC  1200
Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala Ser Val His
385                 390                 395                 400

ACC CTG CTG GAT GCC TTG GAG ACG CTG GGA GAG AGA CTT GCC AAG CAG  1248
Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln
                405                 410                 415

AAG ATT GAG GAC CAC TTG TTG AGC TCT GGA AAG TTC ATG TAT CTA GAA  1296
Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu
            420                 425                 430

GGT AAT GCA GAC TCT GCC ATG TCC TAA                             1323
Gly Asn Ala Asp Ser Ala Met Ser *
        435                 440
```

FIG. 26B

EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSGINWQGGSTGY
ADSVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

<451 residuos; 0 stop; peso molecular: 49167.50

FIG. 27

SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIYGANNRPSGIPDRF
SGSSSGNTASLTITGAQAEDEADYYCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS
LTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

<213 residuos; 0 stop; peso molecular: 22400.70

FIG. 28

Alineación, Cadenas Pesadas 16ES y Apomab 7.3

```
16E2           1 EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYGMSW
                                          *
Apomab7.3      1 EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSW

16E2          37 VRQAPGKGLEWVSGINWNGGSTGYADSVKGRVTISRDNAKNSLYLQMNSL
                                 *
Apomab7.3     37 VRQAPGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLYLQMNSL

16E2          87 RAEDTAVYYCAKILGAGRGWYFDLWGKGTTVTVSSASTKGPSVFPLAPSS
                                       *
Apomab7.3     87 RAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTVSSASTKGPSVFPLAPSS

16E2         137 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
Apomab7.3    137 KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL

16E2         187 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
Apomab7.3    187 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP

16E2         237 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
Apomab7.3    237 ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV

16E2         287 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
Apomab7.3    287 EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI

16E2         337 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE
Apomab7.3    337 EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE

16E2         387 SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
Apomab7.3    387 SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

16E2.huIgG1  437 HNHYTQKSLSLSPGK
Apomab7.3    437 HNHYTQKSLSLSPGK
```

FIG. 29

Alineación, Cadenas Ligeras 16E2 y Apomab 7.3

```
16E2          1 SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKN
                                *                       *
Apomab7.3     1 SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIYGAN

16E2         51 NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGG
                                                    *
Apomab7.3    51 NRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSADSSGNHVVFGG

16E2        101 GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK
Apomab7.3   101 GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK

16E2        151 ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEG
Apomab7.3   151 ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEG

16E2        201 STVEKTVAPTECS
Apomab7.3   201 STVEKTVAPTECS
```

FIG. 30

Secuencia de Dominio Pesado Variable de Apomab

CDR H1
EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMWVRQAPGKGLEWV

CDR H2
SGIRWQGGSTGYADSVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAK

CDR H3
ILGAGRGWYFDYWGKGTTVTVSSASTKGP

FIG. 31A

Secuencia de Dominio Ligero Variable de Apomab

CDR L1
SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQAPVLVIY

CDR L2 CDRL3
GANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSADSSGNHVV

FGGGTKLTVLG

FIG. 31B

Alineación de Cadena Ligera

```
                1                                32
hu2H7.v16       DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP
                ********************************.*******************
hu2H7.v511      DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP

               52
hu2H7.v16       SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG
                ********************************************.**********
hu2H7.v511      SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG

              102
hu2H7.v16       TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
                ************************************************** 
hu2H7.v511      TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD

              152
hu2H7.v16       NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL
                **************************************************
hu2H7.v511      NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL

              202         214
hu2H7.v16       SSPVTKSFNRGEC
                *************
hu2H7.v511      SSPVTKSFNRGEC
```

FIG. 32

Alineación de Cadena Pesada

```
                1
hu2H7.v16       EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYHMHW
                ************************************
hu2H7.v511      EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYHMHW

               37              52a                                82abc
hu2H7.v16       VRQAPGKGLEWVGAIYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSL
                ********************* ********************************
hu2H7.v511      VRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL

               83                100abcde          113
hu2H7.v16       RAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS
                ******************  ******************
hu2H7.v511      RAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS

              118
hu2H7.v16       ASTKGPSVFPLAPS
                **************
hu2H7.v511      ASTKGPSVFPLAPS

              132
hu2H7.v16       SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
                ************************************************
hu2H7.v511      SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS

              182
hu2H7.v16       LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
                ************************************************
hu2H7.v511      LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA

              232
hu2H7.v16       PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
                ************************************************
hu2H7.v511      PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG

              282
hu2H7.v16       VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
                ****************.*********************************
hu2H7.v511      VEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAP

              332
hu2H7.v16       IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
                *  ************************************************
hu2H7.v511      IAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW

              382
hu2H7.v16       ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
                ************************************************
hu2H7.v511      ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

              432             447
hu2H7.v16       LHNHYTQKSLSLSPGK
                ****************
hu2H7.v511      LHNHYTQKSLSLSPGK
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FIG. 33

Listado de Secuencias

<110> GENENTECH, INC.
ANDYA, JAMES
GWEE, SHIANG C.
LIU, JUN
SHEN,YE

<120> FORMULACIONES DE ANTICUERPO

<130> P2104R1 PCT

<140> PCT/US2005/037471
<141> 2005-10-19

<150> US 60/620,413
<151> 2004-10-20

<160> 74

<210> 1
<211> 107
<212> PRT
<213> Mus musculus

<400> 1

Asp	Thr	Val	Met	Thr	Gln	Ser	His	Lys	Ile	Met	Ser	Thr	Ser	Val	
1				5				10						15	
Gly	Asp	Arg	Val	Ser	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp	Val	Ser	
				20				25						30	
Ile	Gly	Val	Ala	Trp	Tyr	Gln	Gln	Arg	Pro	Gly	Gln	Ser	Pro	Lys	
				35				40						45	
Leu	Leu	Ile	Tyr	Ser	Ala	Ser	Tyr	Arg	Tyr	Thr	Gly	Val	Pro	Asp	
				50				55						60	
Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	Thr	Ile	
				65				70						75	
Ser	Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	
				80				85						90	
Tyr	Tyr	Ile	Tyr	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	
				95				100						105	
Ile	Lys														

<210> 2
<211> 119
<212> PRT
<213> Mus musculus

<400> 2

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	
1				5				10						15	
Thr	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Phe	Thr	Phe	Thr	
				20				25						30	
Asp	Tyr	Thr	Met	Asp	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	
				35				40						45	
Glu	Trp	Ile	Gly	Asp	Val	Asn	Pro	Asn	Ser	Gly	Gly	Ser	Ile	Tyr	
				50				55						60	
Asn	Gln	Arg	Phe	Lys	Gly	Lys	Ala	Ser	Leu	Thr	Val	Asp	Arg	Ser	
				65				70						75	
Ser	Arg	Ile	Val	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Phe	Glu	Asp	
				80				85						90	
Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asn	Leu	Gly	Pro	Ser	Phe	Tyr	
				95				100						105	

Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110 115

<210> 3
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 3
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 4
<211> 119
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 4
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
50 55 60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
95 100 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 5
<211> 107
<212> PRT
<213> Secuencia artificial

<220>

<223> Se sintetiza la secuencia

<400> 5
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30

Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 6
<211> 119
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 6
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
95 100 105

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 7
<211> 10
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 10
<223> Xaa es preferiblemente D o S

<400> 7
Gly Phe Thr Phe Thr Asp Tyr Thr Met Xaa
5 10

<210> 8
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 8
Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr Asn Gln Arg Phe
1 5 10 15

Lys Gly

<210> 9
<211> 10
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 9
Asn Leu Gly Pro Ser Phe Tyr Phe Asp Tyr
5 10

<210> 10
<211> 11
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 10
Lys Ala Ser Gln Asp Val Ser Ile Gly Val Ala
5 10

<210> 11
<211> 7
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 5
<223> Xaa es preferiblemente R o L

<220>
<221> Xaa
<222> 6
<223> Xaa es preferiblemente Y o E

<220>
<221> Xaa
<222> 7
<223> Xaa es preferiblemente T o S

<400> 11
Ser Ala Ser Tyr Xaa Xaa Xaa
5

<210> 12
<211> 9
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr
5

<210> 13
<211> 214
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 13
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn
20 25 30

Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 14
<211> 449
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 14
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Thr Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr

```
                50                  55                  60

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

Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ser Arg Trp Gly Gly Asp Gly Phe Tyr
                95                 100                 105

Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
               110                 115                 120

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
               125                 130                 135

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
               140                 145                 150

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
               155                 160                 165

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
               170                 175                 180

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
               185                 190                 195

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
               200                 205                 210

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
               215                 220                 225

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
               230                 235                 240

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

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
               260                 265                 270

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
               275                 280                 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
               290                 295                 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
               305                 310                 315

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
               320                 325                 330

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
               335                 340                 345

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
               350                 355                 360

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
               365                 370                 375

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
               380                 385                 390

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
               395                 400                 405

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
               410                 415                 420

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
               425                 430                 435
```

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445

<210> 15
<211> 214
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 15
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser
20 25 30

Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 16
<211> 448
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 16
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

```
Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
                50                  55                  60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
                65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
                95                 100                 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
               110                 115                 120

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
               125                 130                 135

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
               140                 145                 150

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
               155                 160                 165

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
               170                 175                 180

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
               185                 190                 195

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
               200                 205                 210

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
               215                 220                 225

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
               230                 235                 240

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

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
               260                 265                 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
               275                 280                 285

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

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
               305                 310                 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
               320                 325                 330

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
               335                 340                 345

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
               350                 355                 360

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
               365                 370                 375

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
               380                 385                 390

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
               395                 400                 405

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
               410                 415                 420

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
```

425 430 435

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445

<210> 17
<211> 233
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 17
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
20 25 30

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

Gln Asp Val Ser Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly
50 55 60

Lys Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr
65 70 75

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
80 85 90

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
95 100 105

Tyr Cys Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly
110 115 120

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
125 130 135

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
140 145 150

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
155 160 165

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
170 175 180

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
185 190 195

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
200 205 210

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
215 220 225

Lys Ser Phe Asn Arg Gly Glu Cys
230

<210> 18
<211> 467
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 18
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

Gly Val His Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Thr Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro
50 55 60

Gly Lys Gly Leu Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly
65 70 75

Gly Ser Ile Tyr Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser
80 85 90

Val Asp Arg Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
95 100 105

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly
110 115 120

Pro Ser Phe Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
125 130 135

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
140 145 150

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
155 160 165

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
170 175 180

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
185 190 195

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
200 205 210

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
215 220 225

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
230 235 240

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245 250 255

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
275 280 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
290 295 300

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305 310 315

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
320 325 330

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
335 340 345

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
350 355 360

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
365 370 375

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
380 385 390

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
395 400 405

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
410 415 420

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
425 430 435

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
440 445 450

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
455 460 465

Pro Gly

<210> 19
<211> 195
<212> PRT
<213> Homo sapiens

<400> 19
Thr Gln Val Cys Thr Gly Thr Asp Met Lys Leu Arg Leu Pro Ala
1 5 10 15

Ser Pro Glu Thr His Leu Asp Met Leu Arg His Leu Tyr Gln Gly
20 25 30

Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr Leu Pro Thr
35 40 45

Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val Gln Gly
50 55 60

Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu Gln
65 70 75

Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
80 85 90

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr
95 100 105

Pro Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu
110 115 120

Arg Ser Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg
125 130 135

Asn Pro Gln Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile
140 145 150

Phe His Lys Asn Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn
155 160 165

Arg Ser Arg Ala Cys His Pro Cys Ser Pro Met Cys Lys Gly Ser
170 175 180

Arg Cys Trp Gly Glu Ser Ser Glu Asp Cys Gln Ser Leu Thr Arg
185 190 195

<210> 20
<211> 124
<212> PRT
<213> Homo sapiens

<400> 20
Thr Val Cys Ala Gly Gly Cys Ala Arg Cys Lys Gly Pro Leu Pro
1 5 10 15

Thr Asp Cys Cys His Glu Gln Cys Ala Ala Gly Cys Thr Gly Pro
20 25 30

Lys His Ser Asp Cys Leu Ala Cys Leu His Phe Asn His Ser Gly
35 40 45

Ile Cys Glu Leu His Cys Pro Ala Leu Val Thr Tyr Asn Thr Asp

50 55 60

Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg Tyr Thr Phe Gly
65 70 75

Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu Ser Thr Asp
80 85 90

Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln Glu Val
95 100 105

Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys Pro
110 115 120

Cys Ala Arg Val

<210> 21
<211> 169
<212> PRT
<213> Homo sapiens

<400> 21
Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu Val Arg Ala Val
1 5 10 15

Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys Lys Ile Phe
20 25 30

Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp Pro Ala
35 40 45

Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe Glu
50 55 60

Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
65 70 75

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile
80 85 90

Arg Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln
95 100 105

Gly Leu Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu
110 115 120

Gly Ser Gly Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe
125 130 135

Val His Thr Val Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln
140 145 150

Ala Leu Leu His Thr Ala Asn Arg Pro Glu Asp Glu Cys Val Gly
155 160 165

Glu Gly Leu Ala

<210> 22
<211> 142
<212> PRT
<213> Homo sapiens

<400> 22
Cys His Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro
1 5 10 15

Thr Gln Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys
20 25 30

Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val
35 40 45

Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln
50 55 60

```
Asn Gly Ser Val Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val
                65                  70                  75

Ala Cys Ala His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg Cys
                80                  85                  90

Pro Ser Gly Val Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys
                95                 100                 105

Phe Pro Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn Cys
               110                 115                 120

Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly Cys Pro Ala Glu
               125                 130                 135

Gln Arg Ala Ser Pro Leu Thr
               140
```

<210> 23
<211> 217
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 23

```
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
  1                 5                  10                  15

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln
                20                  25                  30

Asp Val Ser Ile Gly Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys
                35                  40                  45

Ala Pro Lys Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly
            50                  55                  60

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                65                  70                  75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
                80                  85                  90

Cys Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr Phe Gly Gln Gly Thr
                95                 100                 105

Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile
               110                 115                 120

Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
               125                 130                 135

Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
               140                 145                 150

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
               155                 160                 165

Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
               170                 175                 180

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
               185                 190                 195

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
               200                 205                 210

Ser Phe Asn Arg Gly Glu Cys
               215
```

<210> 24
<211> 449
<212> PRT

<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 24

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

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

Asp Tyr Thr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
             35                  40                  45

Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
             50                  55                  60

Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
             65                  70                  75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
             80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asn Leu Gly Pro Ser Phe Tyr
             95                 100                 105

Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
            110                 115                 120

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
            125                 130                 135

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
            140                 145                 150

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
        155                 160                 165

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
            170                 175                 180

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
            185                 190                 195

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
            200                 205                 210

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
            215                 220                 225

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
            230                 235                 240

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

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            260                 265                 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
            275                 280                 285

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

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            305                 310                 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            320                 325                 330

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
            335                 340                 345

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
```

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
365 370 375

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
380 385 390

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
395 400 405

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
410 415 420

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
425 430 435

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
440 445

<210> 25
<211> 107
<212> PRT
<213> Mus musculus

<400> 25
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro
1 5 10 15

Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro
35 40 45

Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser
65 70 75

Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
95 100 105

Lys Arg

<210> 26
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 26
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

Ser Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105

Lys Arg

<210> 27
<211> 108
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 27
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30

Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg

<210> 28
<211> 122
<212> PRT
<213> Mus musculus

<400> 28
Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly
1 5 10 15

Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu
35 40 45

Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
65 70 75

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp
80 85 90

Ser Ala Val Tyr Phe Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr Val
110 115 120

Ser Ser

<210> 29
<211> 122
<212> PRT

<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser

<210> 30
<211> 119
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 30
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Val Gly Tyr Ser Leu
95 100 105

Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

<210> 31
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 31
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu

```
  1               5                  10                  15

Gly Asp Arg Val Ile Ile Ser Cys Ser Ala Ser Gln Asp Ile Ser
                20                  25                  30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys
                35                  40                  45

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                65                  70                  75

Ser Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
                95                  100                 105

Ile Lys
```

<210> 32
<211> 123
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

```
<400> 32
 Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Gln Pro Gly
   1               5                  10                  15

 Glu Thr Val Arg Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
                 20                  25                  30

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

 Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
                 50                  55                  60

 Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Glu Thr Ser
                 65                  70                  75

 Ala Ser Thr Ala Tyr Leu Gln Ile Ser Asn Leu Lys Asn Asp Asp
                 80                  85                  90

 Thr Ala Thr Tyr Phe Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
                 95                  100                 105

 Ser His Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr
                110                  115                 120

 Val Ser Ser
```

<210> 33
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

```
<400> 33
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
   1               5                  10                  15

 Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser
                 20                  25                  30

 Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                 35                  40                  45
```

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 34
<211> 123
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 34
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
50 55 60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
65 70 75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser
95 100 105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser

<210> 35
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 35
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser
20 25 30

Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Val Leu Ile Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Ser Thr Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys

<210> 36
<211> 123
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 36
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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

Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr
50 55 60

Ala Ala Asp Phe Lys Arg Arg Phe Thr Phe Ser Leu Asp Thr Ser
65 70 75

Lys Ser Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro Tyr Tyr Tyr Gly Thr
95 100 105

Ser His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser

<210> 37
<211> 218
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 37
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp
20 25 30

Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly
35 40 45

Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser
50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
80 85 90

Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly
95 100 105

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe

110 115 120

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
125 130 135

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
140 145 150

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
155 160 165

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
170 175 180

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
185 190 195

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
200 205 210

Lys Ser Phe Asn Arg Gly Glu Cys
215

<210> 38
<211> 218
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 38
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Pro Val Asp
20 25 30

Gly Glu Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
35 40 45

Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser
50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
80 85 90

Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly
95 100 105

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
110 115 120

Ile Phe Pro Pro Ser Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
125 130 135

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
140 145 150

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
155 160 165

Ser Phe Asn Arg Gly Glu Cys Asp Glu Gln Leu Lys Ser Gly Thr
170 175 180

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
185 190 195

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
200 205 210

Gln Glu Ser Val Thr Glu Gln Asp
215

<210> 39
<211> 218
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 39

```
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
  1               5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Pro Val Asp
             20                  25                  30

Gly Glu Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly
             35                  40                  45

Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser
             50                  55                  60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
             65                  70                  75

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
             80                  85                  90

Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly
             95                 100                 105

Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
            110                 115                 120

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
            125                 130                 135

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
            140                 145                 150

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
            155                 160                 165

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
            170                 175                 180

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            185                 190                 195

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
            200                 205                 210

Lys Ser Phe Asn Arg Gly Glu Cys
            215
```

<210> 40
<211> 214
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 40

```
Asp Ile Leu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro
  1               5                  10                  15

Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Gly
             20                  25                  30

Thr Asn Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
             35                  40                  45

Leu Leu Ile Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser
             50                  55                  60
```

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
65 70 75

Ser Arg Leu Glu Pro Glu Asp Phe Ala Met Tyr Tyr Cys Gln Gln
80 85 90

Ser Asp Ser Trp Pro Thr Thr Phe Gly Gln Gly Thr Lys Val Glu
95 100 105

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
140 145 150

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
155 160 165

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
170 175 180

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
185 190 195

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
200 205 210

Arg Gly Glu Cys

<210> 41
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 41
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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

Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr
50 55 60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
95 100 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
110 115 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser

170 175 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185 190 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200 205 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215 220 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
320 325 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
335 340 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350 355 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365 370 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380 385 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395 400 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410 415 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 42
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 42
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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

```
Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr
                50                  55                  60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
                95                 100                 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
               110                 115                 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
               125                 130                 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
               140                 145                 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
               155                 160                 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
               170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
               185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
               200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
               215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
               230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
               245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
               260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
               275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
               290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
               305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
               320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
               335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
               350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
               365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
               380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
               395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
               410                 415                 420
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Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 43
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 43
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr
20 25 30

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

Leu Glu Trp Val Ala Ser Ile Lys Tyr Ser Gly Glu Thr Lys Tyr
50 55 60

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

Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His
95 100 105

Trp His Phe Ala Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
110 115 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
170 175 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185 190 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200 205 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215 220 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr

290 295 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
320 325 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
335 340 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350 355 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365 370 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380 385 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395 400 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410 415 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

<210> 44
<211> 453
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 44
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser
20 25 30

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

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

Asn Glu Lys Phe Lys Ala Arg Ala Thr Phe Thr Ala Asp Thr Ser
65 70 75

Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Phe Ser His Phe Ser Gly Ser
95 100 105

Asn Tyr Asp Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
125 130 135

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
140 145 150

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
155 160 165

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
170 175 180

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
185 190 195

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
200 205 210

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
215 220 225

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
230 235 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
275 280 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
290 295 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
305 310 315

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
320 325 330

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
335 340 345

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
350 355 360

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
365 370 375

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
380 385 390

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
395 400 405

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
410 415 420

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
425 430 435

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
440 445 450

Pro Gly Lys

<210> 45
<211> 1042
<212> DNA
<213> Homo sapiens

<220>
<221> Unsure
<222> 447
<223> N es T o G

<400> 45
tttcctcact gactataaaa gaatagagaa ggaagggctt cagtgaccgg 50

ctgcctggct gacttacagc agtcagactc tgacaggatc atggctatga 100

tggaggtcca gggggaccc agcctgggac agacctgcgt gctgatcgtg 150

atcttcacag tgctcctgca gtctctctgt gtggctgtaa cttacgtgta 200

ctttaccaac gagctgaagc agatgcagga caagtactcc aaaagtggca 250

ttgcttgttt cttaaagaaa gatgacagtt attgggaccc caatgacgaa 300

gagagtatga acagcccctg ctggcaagtc aagtggcaac tccgtcagct 350

cgttagaaag atgattttga gaacctctga ggaaaccatt tctacagttc 400

aagaaaagca acaaaatatt tctccctag tgagagaaag aggtccncag 450

agagtagcag ctcacataac tgggacaaga ggaagaagca acacattgtc 500

ttctccaaac tccaagaatg aaaaggctct gggccgcaaa ataaactcct 550

gggaatcatc aaggagtggg cattcattcc tgagcaactt gcacttgagg 600

aatggtgaac tggtcatcca tgaaaaaggg ttttactaca tctattccca 650

aacatacttt cgatttcagg aggaaataaa agaaaacaca aagaacgaca 700

aacaaatggt ccaatatatt tacaaataca caagttatcc tgaccctata 750

ttgttgatga aaagtgctag aaatagttgt tggtctaaag atgcagaata 800

tggactctat tccatctatc aaggggggaat atttgagctt aaggaaaatg 850

acagaatttt tgtttctgta acaaatgagc acttgataga catggaccat 900

gaagccagtt ttttcggggc ctttttagtt ggctaactga cctggaaaga 950

aaaagcaata acctcaaagt gactattcag ttttcaggat gatacactat 1000

gaagatgttt caaaaaatct gaccaaaaca aacaaacaga aa 1042

<210> 46
<211> 281
<212> PRT
<213> Homo sapiens

<400> 46

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

Cys Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys
20 25 30

Val Ala Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met
35 40 45

Gln Asp Lys Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu
50 55 60

Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser
65 70 75

Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln Leu Val Arg Lys
80 85 90

Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr Val Gln Glu
95 100 105

Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly Pro Gln
110 115 120

Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn Thr
125 130 135

Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys
140 145 150

Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser
155 160 165

Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly
170 175 180

Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu
185 190 195

Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile
200 205 210

Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser
215 220 225

Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
230 235 240

Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg
245 250 255

Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His
260 265 270

Glu Ala Ser Phe Phe Gly Ala Phe Leu Val Gly
275 280

<210> 47
<211> 411
<212> PRT
<213> Homo sapiens

<220>
<221> Xaa
<222> 410
<223> Xaa es L o M

<400> 47
Met Glu Gln Arg Gly Gln Asn Ala Pro Ala Ala Ser Gly Ala Arg
1 5 10 15

Lys Arg His Gly Pro Gly Pro Arg Glu Ala Arg Gly Ala Arg Pro
20 25 30

Gly Leu Arg Val Pro Lys Thr Leu Val Leu Val Val Ala Ala Val
35 40 45

Leu Leu Leu Val Ser Ala Glu Ser Ala Leu Ile Thr Gln Gln Asp
50 55 60

Leu Ala Pro Gln Gln Arg Ala Ala Pro Gln Gln Lys Arg Ser Ser
65 70 75

Pro Ser Glu Gly Leu Cys Pro Pro Gly His His Ile Ser Glu Asp
80 85 90

Gly Arg Asp Cys Ile Ser Cys Lys Tyr Gly Gln Asp Tyr Ser Thr
95 100 105

His Trp Asn Asp Leu Leu Phe Cys Leu Arg Cys Thr Arg Cys Asp
110 115 120

Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr
125 130 135

Val Cys Gln Cys Glu Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro
140 145 150

Glu Met Cys Arg Lys Cys Arg Thr Gly Cys Pro Arg Gly Met Val
155 160 165

Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His
170 175 180

Lys Glu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
185 190 195

Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys

200 205 210

Val Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp
215 220 225

Pro Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp
230 235 240

Asn Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val
245 250 255

Pro Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly
260 265 270

Val Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro
275 280 285

Ala Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala
290 295 300

Asn Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp
305 310 315

Phe Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg
320 325 330

Lys Leu Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu
335 340 345

Ala Ala Gly His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp
350 355 360

Val Asn Lys Thr Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp
365 370 375

Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu
380 385 390

Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn
395 400 405

Ala Asp Ser Ala Xaa Ser
410

<210> 48
<211> 1799
<212> DNA
<213> Homo sapiens

<400> 48
cccacgcgtc cgcataaatc agcacgcggc cggagaaccc cgcaatctct 50

gcgcccacaa aatacaccga cgatgcccga tctactttaa gggctgaaac 100

ccacgggcct gagagactat aagagcgttc cctaccgcca tggaacaacg 150

gggacagaac gccccggccg cttcgggggc ccggaaaagg cacggcccag 200

gacccaggga ggcgcgggga gccaggcctg ggctccgggt ccccaagacc 250

cttgtgctcg ttgtcgccgc ggtcctgctg ttggtctcag ctgagtctgc 300

tctgatcacc caacaagacc tagctccccc gcagagagcg gccccacaac 350

aaaagaggtc cagccctca gagggattgt gtccacctgg acaccatatc 400

tcagaagacg gtagagattg catctcctgc aaatatggac aggactatag 450

cactcactgg aatgacctcc ttttctgctt gcgctgcacc aggtgtgatt 500

caggtgaagt ggagctaagt ccctgcacca cgaccagaaa cacagtgtgt 550

cagtgcgaag aaggcacctt ccgggaagaa gattctcctg agatgtgccg 600

gaagtgccgc acagggtgtc ccagagggat ggtcaaggtc ggtgattgta 650

caccctggag tgacatcgaa tgtgtccaca aagaatcagg catcatcata 700

ggagtcacag ttgcagccgt agtcttgatt gtggctgtgt ttgtttgcaa 750

gtctttactg tggaagaaag tccttcctta cctgaaaggc atctgctcag 800

gtggtggtgg ggaccctgag cgtgtggaca gaagctcaca acgacctggg 850

gctgaggaca atgtcctcaa tgagatcgtg agtatcttgc agcccaccca 900

ggtccctgag caggaaatgg aagtccagga gccagcagag ccaacaggtg 950

tcaacatgtt gtcccccggg gagtcagagc atctgctgga accggcagaa 1000

gctgaaaggt ctcagaggag gaggctgctg gttccagcaa atgaaggtga 1050

tcccactgag actctgagac agtgcttcga tgactttgca gacttggtgc 1100

cctttgactc ctgggagccg ctcatgagga agttgggcct catggacaat 1150

gagataaagg tggctaaagc tgaggcagcg ggccacaggg acaccttgta 1200

cacgatgctg ataaagtggg tcaacaaaac cgggcgagat gcctctgtcc 1250

acaccctgct ggatgccttg gagacgctgg gagagagact tgccaagcag 1300

aagattgagg accacttgtt gagctctgga aagttcatgt atctagaagg 1350

taatgcagac tctgccwtgt cctaagtgtg attctcttca ggaagtgaga 1400

ccttccctgg tttacctttt ttctggaaaa agcccaactg gactccagtc 1450

agtaggaaag tgccacaatt gtcacatgac cggtactgga agaaactctc 1500

ccatccaaca tcacccagtg gatggaacat cctgtaactt ttcactgcac 1550

ttggcattat ttttataagc tgaatgtgat aataaggaca ctatggaaat 1600

gtctggatca ttccgtttgt gcgtactttg agatttggtt tgggatgtca 1650

ttgttttcac agcacttttt tatcctaatg taaatgcttt atttatttat 1700

ttgggctaca ttgtaagatc catctacaaa aaaaaaaaaa aaaaaaaaag 1750

ggcggccgcg actctagagt cgacctgcag aagcttggcc gccatggcc 1799

<210> 49
<211> 440
<212> PRT
<213> Homo sapiens

<400> 49

Met	Glu	Gln	Arg	Gly	Gln	Asn	Ala	Pro	Ala	Ala	Ser	Gly	Ala	Arg	
1				5				10						15	
Lys	Arg	His	Gly	Pro	Gly	Pro	Arg	Glu	Ala	Arg	Gly	Ala	Arg	Pro	
				20				25						30	
Gly	Pro	Arg	Val	Pro	Lys	Thr	Leu	Val	Leu	Val	Val	Ala	Ala	Val	
				35				40						45	
Leu	Leu	Leu	Val	Ser	Ala	Glu	Ser	Ala	Leu	Ile	Thr	Gln	Gln	Asp	
				50				55						60	
Leu	Ala	Pro	Gln	Gln	Arg	Ala	Ala	Pro	Gln	Gln	Lys	Arg	Ser	Ser	
				65				70						75	
Pro	Ser	Glu	Gly	Leu	Cys	Pro	Pro	Gly	His	His	Ile	Ser	Glu	Asp	
				80				85						90	
Gly	Arg	Asp	Cys	Ile	Ser	Cys	Lys	Tyr	Gly	Gln	Asp	Tyr	Ser	Thr	
				95				100						105	
His	Trp	Asn	Asp	Leu	Leu	Phe	Cys	Leu	Arg	Cys	Thr	Arg	Cys	Asp	
				110				115						120	

Ser Gly Glu Val Glu Leu Ser Pro Cys Thr Thr Thr Arg Asn Thr
125 130 135

Val Cys Gln Cys Glu Glu Gly Thr Phe Arg Glu Glu Asp Ser Pro
140 145 150

Glu Met Cys Arg Lys Cys Arg Thr Gly Cys Pro Arg Gly Met Val
155 160 165

Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile Glu Cys Val His
170 175 180

Lys Glu Ser Gly Thr Lys His Ser Gly Glu Ala Pro Ala Val Glu
185 190 195

Glu Thr Val Thr Ser Ser Pro Gly Thr Pro Ala Ser Pro Cys Ser
200 205 210

Leu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val Leu
215 220 225

Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys Val
230 235 240

Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp Pro
245 250 255

Glu Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp Asn
260 265 270

Val Leu Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro
275 280 285

Glu Gln Glu Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val
290 295 300

Asn Met Leu Ser Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala
305 310 315

Glu Ala Glu Arg Ser Gln Arg Arg Arg Leu Leu Val Pro Ala Asn
320 325 330

Glu Gly Asp Pro Thr Glu Thr Leu Arg Gln Cys Phe Asp Asp Phe
335 340 345

Ala Asp Leu Val Pro Phe Asp Ser Trp Glu Pro Leu Met Arg Lys
350 355 360

Leu Gly Leu Met Asp Asn Glu Ile Lys Val Ala Lys Ala Glu Ala
365 370 375

Ala Gly His Arg Asp Thr Leu Tyr Thr Met Leu Ile Lys Trp Val
380 385 390

Asn Lys Thr Gly Arg Asp Ala Ser Val His Thr Leu Leu Asp Ala
395 400 405

Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln Lys Ile Glu Asp
410 415 420

His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu Gly Asn Ala
425 430 435

Asp Ser Ala Met Ser
440

<210> 50
<211> 1323
<212> DNA
<213> Homo sapiens

<400> 50
atggaacaac ggggacagaa cgccccggcc gcttcggggg cccggaaaag 50

gcacggccca ggacccaggg aggcgcgggg agccaggcct gggccccggg 100

tccccaagac ccttgtgctc gttgtcgccg cggtcctgct gttggtctca 150

gctgagtctg ctctgatcac ccaacaagac ctagctcccc agcagagagc 200

ggccccacaa caaaagaggt ccagcccctc agagggattg tgtccacctg 250

gacaccatat ctcagaagac ggtagagatt gcatctcctg caaatatgga 300

caggactata gcactcactg gaatgacctc cttttctgct tgcgctgcac 350

caggtgtgat tcaggtgaag tggagctaag tccgtgcacc acgaccagaa 400

acacagtgtg tcagtgcgaa gaaggcacct tccgggaaga agattctcct 450

gagatgtgcc ggaagtgccg cacagggtgt cccagaggga tggtcaaggt 500

cggtgattgt acaccctgga gtgacatcga atgtgtccac aaagaatcag 550

gtacaaagca cagtggggaa gccccagctg tggaggagac ggtgacctcc 600

agcccaggga ctcctgcctc tccctgttct ctctcaggca tcatcatagg 650

agtcacagtt gcagccgtag tcttgattgt ggctgtgttt gtttgcaagt 700

ctttactgtg gaagaaagtc cttccttacc tgaaaggcat ctgctcaggt 750

ggtggtgggg accctgagcg tgtggacaga agctcacaac gacctggggc 800

tgaggacaat gtcctcaatg agatcgtgag tatcttgcag cccacccagg 850

tccctgagca ggaaatggaa gtccaggagc cagcagagcc aacaggtgtc 900

aacatgttgt ccccgggga gtcagagcat ctgctggaac cggcagaagc 950

tgaaaggtct cagaggagga ggctgctggt tccagcaaat gaaggtgatc 1000

ccactgagac tctgagacag tgcttcgatg actttgcaga cttggtgccc 1050

tttgactcct gggagccgct catgaggaag ttgggcctca tggacaatga 1100

gataaaggtg gctaaagctg aggcagcggg ccacagggac accttgtaca 1150

cgatgctgat aaagtgggtc aacaaaaccg ggcgagatgc ctctgtccac 1200

accctgctgg atgccttgga gacgctggga gagagacttg ccaagcagaa 1250

gattgaggac cacttgttga gctctggaaa gttcatgtat ctagaaggta 1300

atgcagactc tgccatgtcc taa 1323

:210> 51
:211> 451
:212> PRT
:213> Secuencia artificial

:220>
:223> Se sintetiza la secuencia

:400> 51

Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Gly	Gly	Val	Glu	Arg	Pro	Gly	
1				5				10						15	
Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	
				20				25						30	
Asp	Tyr	Ala	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
				35				40						45	
Glu	Trp	Val	Ser	Gly	Ile	Asn	Trp	Gln	Gly	Gly	Ser	Thr	Gly	Tyr	
				50				55						60	
Ala	Asp	Ser	Val	Lys	Gly	Arg	Val	Thr	Ile	Ser	Arg	Asp	Asn	Ala	
				65				70						75	
Lys	Asn	Ser	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	
				80				85						90	

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Thr Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly
                 95                  100                 105

Trp Tyr Phe Asp Tyr Trp Gly Lys Gly Thr Thr Val Thr Val Ser
                110                 115                 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
                125                 130                 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
                140                 145                 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
                155                 160                 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
                200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
                215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
                290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
                350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
                380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                410                 415                 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                425                 430                 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
                440                 445                 450

Lys
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<210> 52
<211> 213
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 52
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Ser Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Ala Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Ala Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro
110 115 120

Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
125 130 135

Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
140 145 150

Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro
155 160 165

Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser
170 175 180

Leu Thr Pro Glu Gln Trp Lys Ser His Lys Ser Tyr Ser Cys Gln
185 190 195

Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr
200 205 210

Glu Cys Ser

<210> 53
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<220>
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Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
20 25 30

Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr
50 55 60

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

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                65                  70                  75

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
            80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly
        95                  100                 105

Trp Tyr Phe Asp Leu Trp Gly Lys Gly Thr Thr Val Thr Val Ser
    110                 115                 120

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125                 130                 135

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140                 145                 150

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155                 160                 165

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
170                 175                 180

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185                 190                 195

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200                 205                 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215                 220                 225

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230                 235                 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245                 250                 255

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
        260                 265                 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290                 295                 300

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305                 310                 315

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
320                 325                 330

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
335                 340                 345

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350                 355                 360

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365                 370                 375

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380                 385                 390

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395                 400                 405

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410                 415                 420

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425                 430                 435

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
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Lys

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<211> 213
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<223> Se sintetiza la secuencia

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Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro
110 115 120

Pro Ser Ser Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys
125 130 135

Leu Ile Ser Asp Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys
140 145 150

Ala Asp Ser Ser Pro Val Lys Ala Gly Val Glu Thr Thr Thr Pro
155 160 165

Ser Lys Gln Ser Asn Asn Lys Tyr Ala Ala Ser Ser Tyr Leu Ser
170 175 180

Leu Thr Pro Glu Gln Trp Lys Ser His Lys Ser Tyr Ser Cys Gln
185 190 195

Val Thr His Glu Gly Ser Thr Val Glu Lys Thr Val Ala Pro Thr
200 205 210

Glu Cys Ser

<210> 55
<211> 126
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<220>
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<400> 55
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1 5 10 15

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
20 25 30

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

Trp Val Ser Gly Ile Asn Trp Gln Gly Gly Ser Thr Gly Tyr Ala
50 55 60

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

Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
80 85 90

Ala Val Tyr Tyr Cys Ala Lys Ile Leu Gly Ala Gly Arg Gly Trp
95 100 105

Tyr Phe Asp Tyr Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
110 115 120

Ala Ser Thr Lys Gly Pro
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<210> 56
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<400> 56
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln
1 5 10 15

Thr Val Arg Ile Thr Cys Ser Gly Asp Ser Leu Arg Ser Tyr Tyr
20 25 30

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

Ile Tyr Gly Ala Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
50 55 60

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly
65 70 75

Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Ala Asp
80 85 90

Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
95 100 105

Val Leu Gly

<210> 57
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<212> PRT
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Arg Ala Ser Ser Ser Val Ser Tyr Met His
5 10

<210> 58
<211> 7
<212> PRT
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<220>
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<400> 58
Ala Pro Ser Asn Leu Ala Ser
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<210> 59

<211> 9
<212> PRT
<213> Secuencia artificial

<220>
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<400> 59
Gln Gln Trp Ser Phe Asn Pro Pro Thr
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<210> 60
<211> 10
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<220>
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<400> 60
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
5 10

<210> 61
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 61
Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
1 5 10 15

Lys Gly

<210> 62
<211> 13
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<400> 62
Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
5 10

<210> 63
<211> 213
<212> PRT
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<220>
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<400> 63
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

Ser Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
110 115 120

Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
125 130 135

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
140 145 150

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
155 160 165

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
170 175 180

Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
185 190 195

Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
200 205 210

Gly Glu Cys

<210> 64
<211> 213
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 64
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

Ala Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
110 115 120

Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
125 130 135

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
140 145 150

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
155 160 165

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
170 175 180

Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
185 190 195

Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
200 205 210

Gly Glu Cys

<210> 65
<211> 452
<212> PRT
<213> Secuencia artificial

<220>
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<400> 65
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His

305 310 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
320 325 330

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
335 340 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
350 355 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
365 370 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
380 385 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
395 400 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
410 415 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
440 445 450

Gly Lys

<210> 66
<211> 452
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 66
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305 310 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
320 325 330

Ala Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys
335 340 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
350 355 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
365 370 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
380 385 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
395 400 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
410 415 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
440 445 450

Gly Lys

<210> 67
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<220>
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<220>
<221> Xaa
<222> 9
<223> Xaa es M o L

<400> 67
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<210> 68

<211> 9
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<220>
<223> Se sintetiza la secuencia

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<221> Xaa
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<223> Xaa es S o A

<400> 68
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<210> 69
<211> 17
<212> PRT
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<223> Xaa es D o A

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Lys Gly

<210> 70
<211> 13
<212> PRT
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<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 6
<223> Xaa es N, A, Y, W o D

<220>
<221> Xaa
<222> 7
<223> Xaa es S o R

<400> 70
Val Val Tyr Tyr Ser Xaa Xaa Tyr Trp Tyr Phe Asp Val
5 10

<210> 71
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 71
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

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Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
                50                  55                  60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser
                95                 100                 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
               110                 115                 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
               125                 130                 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
               140                 145                 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
               155                 160                 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
               170                 175                 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
               185                 190                 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
               200                 205                 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
               215                 220                 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
               230                 235                 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
               245                 250                 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
               260                 265                 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
               275                 280                 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
               290                 295                 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
               305                 310                 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
               320                 325                 330

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
               335                 340                 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
               350                 355                 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
               365                 370                 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
               380                 385                 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
               395                 400                 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
               410                 415                 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
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425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
440 445 450

Gly

<210> 72
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 72
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
50 55 60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
95 100 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
110 115 120

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
125 130 135

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
140 145 150

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
155 160 165

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
170 175 180

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
185 190 195

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
200 205 210

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
215 220 225

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
230 235 240

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305 310 315

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
320 325 330

Ala Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys
335 340 345

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
350 355 360

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
365 370 375

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
380 385 390

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
395 400 405

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
410 415 420

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
425 430 435

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
440 445 450

Gly

<210> 73
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 73
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45

Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90

Ala Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105

Lys Arg

<210> 74
<211> 122
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 74

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

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

Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
        35                  40                  45

Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr
    50                  55                  60

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

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg
            95                  100                 105

Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val
        110                 115                 120

Ser Ser
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PCT/US2005/037471

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K47/18

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/105894 A (MEDIMMUNE, INC; OLIVER, CYNTHIA, N; SHANE, ERICA; ISSAACS, BENJAMIN, S) 24 December 2003 (2003-12-24) page 4 page 20 - page 21	1-79
X	WO 03/106644 A (MEDIMMUNE, INC; OLIVER, CYNTHIA, N; ALLAN, CHRISTIAN, B; CHANG, STEPHE) 24 December 2003 (2003-12-24) page 41 - page 42	1-79

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[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
22 June 2006	10/07/2006

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	Wagner, R

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN BEI ET AL: "Influence of histidine on the stability and physical properties of a fully human antibody in aqueous and solid forms." PHARMACEUTICAL RESEARCH (DORDRECHT), vol. 20, no. 12, December 2003 (2003-12), pages 1952-1960, XP002386671 ISSN: 0724-8741 the whole document	1-79
A	WO 03/009817 A (PROTEIN DESIGN LABS, INC; KAISHEVA, ELIZABET, A; FLORES-NATE, ALENI; G) 6 February 2003 (2003-02-06) the whole document	1-79
A	WO 2004/001007 A (IDEC PHARMACEUTICALS CORPORATION; YANG, TZUNG-HORNG; BACICA, MICHAEL,) 31 December 2003 (2003-12-31) the whole document	1-79
A	WO 97/04801 A (GENENTECH, INC) 13 February 1997 (1997-02-13) figure 4	1-79

405

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/037471

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 45,61,62, 76–79 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:

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. [ ] 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.:

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.

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

Information on patent family members

PCT/US2005/037471

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 03105894	A	24-12-2003	AU	2003245515 A1	31-12-2003
			CA	2489632 A1	24-12-2003
			EP	1531862 A1	25-05-2005
			JP	2006501168 T	12-01-2006
WO 03106644	A	24-12-2003	AU	2003276402 A1	31-12-2003
			CA	2489534 A1	24-12-2003
			EP	1576105 A2	21-09-2005
			JP	2006511448 T	06-04-2006
WO 03009817	A	06-02-2003	CA	2454587 A1	06-02-2003
			EP	1409018 A2	21-04-2004
			JP	2004538287 T	24-12-2004
			MX	PA04000747 A	08-07-2004
WO 2004001007	A	31-12-2003	AU	2003251592 A1	06-01-2004
			CA	2490423 A1	31-12-2003
			CN	1671741 A	21-09-2005
			EP	1551875 A2	13-07-2005
			JP	2005530845 T	13-10-2005
WO 9704801	A	13-02-1997	AU	716785 B2	09-03-2000
			AU	6599296 A	26-02-1997
			BR	9609743 A	02-03-1999
			CA	2226575 A1	13-02-1997
			CN	1191490 A	26-08-1998
			CN	1539505 A	27-10-2004
			EP	0845997 A1	10-06-1998
			IL	122910 A	23-05-2002
			JP	11510170 T	07-09-1999
			NO	980335 A	26-03-1998
			NZ	313503 A	28-01-2000
			NZ	500539 A	30-11-2001

103.401.

407

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. Lee, Wendy M.
1 DNA Way
South San Francisco, CA 94080-4990
UNITED STATES OF AMERICA

Applicant's or agent's file reference: P2104R1	FOR FURTHER ACTION: See paragraphs 1 and 4 below
International application No.: PCT/US2005/037471	International filing date (day/month/year): 19/10/2005
Applicant: GENENTECH, INC.	Date of mailing (day/month/year): 10/07/2006

Calendared/LRS Respond to ISR Deadline Consider Chap II - 10 SEP 06 Due Date Chap II/Written Opinion Dead.

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. 10 OCT 06

   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, Facsimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

   Calendared/LRS IDS Due (PCT Search) 30 Days - 16 AUG 06

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	Catriona Cleere

Form PCT/ISA/220 (October 2005) *(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*, Volume I/A, 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 canceling 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 (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.

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.
P2104R1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2005/037471	19/10/2005	20/10/2004
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 6 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. [X] 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. [ ] Unity of invention is lacking (see Box No III)

4. With regard to the title,
   [ ] the text is approved as submitted by the applicant
   [X] the text has been established by this Authority to read as follows:

   ANTIBODY FORMULATION IN HISTIDINE-ACETATE BUFFER

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. _____
      [ ] as suggested by the applicant
      [ ] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [X] none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

PCT/US2005/037471

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   - [X] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material

   - [X] on paper
   - [X] in electronic form

   c. time of filing/furnishing

   - [ ] contained in the international application as filed
   - [ ] filed together with the international application in electronic form
   - [X] furnished subsequently to this Authority for the purpose of search

2. [X] 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.

3. Additional comments:

PCT/US2005/037471

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K47/18

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/105894 A (MEDIMMUNE, INC; OLIVER, CYNTHIA, N; SHANE, ERICA; ISSAACS, BENJAMIN, S) 24 December 2003 (2003-12-24) page 4 page 20 - page 21	1-79
X	WO 03/106644 A (MEDIMMUNE, INC; OLIVER, CYNTHIA, N; ALLAN, CHRISTIAN, B; CHANG, STEPHE) 24 December 2003 (2003-12-24) page 41 - page 42	1-79

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[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
22 June 2006	10/07/2006

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	Wagner, R

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN BEI ET AL: "Influence of histidine on the stability and physical properties of a fully human antibody in aqueous and solid forms." PHARMACEUTICAL RESEARCH (DORDRECHT), vol. 20, no. 12, December 2003 (2003-12), pages 1952-1960, XP002386671 ISSN: 0724-8741 the whole document	1-79
A	WO 03/009817 A (PROTEIN DESIGN LABS, INC; KAISHEVA, ELIZABET, A; FLORES-NATE, ALENI; G) 6 February 2003 (2003-02-06) the whole document	1-79
A	WO 2004/001007 A (IDEC PHARMACEUTICALS CORPORATION; YANG, TZUNG-HORNG; BACICA, MICHAEL,) 31 December 2003 (2003-12-31) the whole document	1-79
A	WO 97/04801 A (GENENTECH, INC) 13 February 1997 (1997-02-13) figure 4	1-79

INTERNATIONAL SEARCH REPORT

PCT/US2005/037471

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 45,61,62, 76-79 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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

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

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers 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. [ ] 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.:

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.

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

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03105894 A	24-12-2003	AU 2003245515 A1	31-12-2003
		CA 2489632 A1	24-12-2003
		EP 1531862 A1	25-05-2005
		JP 2006501168 T	12-01-2006
WO 03106644 A	24-12-2003	AU 2003276402 A1	31-12-2003
		CA 2489534 A1	24-12-2003
		EP 1576105 A2	21-09-2005
		JP 2006511448 T	06-04-2006
WO 03009817 A	06-02-2003	CA 2454587 A1	06-02-2003
		EP 1409018 A2	21-04-2004
		JP 2004538287 T	24-12-2004
		MX PA04000747 A	08-07-2004
WO 2004001007 A	31-12-2003	AU 2003251592 A1	06-01-2004
		CA 2490423 A1	31-12-2003
		CN 1671741 A	21-09-2005
		EP 1551875 A2	13-07-2005
		JP 2005530845 T	13-10-2005
WO 9704801 A	13-02-1997	AU 716785 B2	09-03-2000
		AU 6599296 A	26-02-1997
		BR 9609743 A	02-03-1999
		CA 2226575 A1	13-02-1997
		CN 1191490 A	26-08-1998
		CN 1539505 A	27-10-2004
		EP 0845997 A1	10-06-1998
		IL 122910 A	23-05-2002
		JP 11510170 T	07-09-1999
		NO 980335 A	26-03-1998
		NZ 313503 A	28-01-2000
		NZ 500539 A	30-11-2001

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/US2005/037471	19.10.2005	20.10.2004

International Patent Classification (IPC) or both national classification and IPC
INV. A61K39/395 A61K47/18

Applicant
GENENTECH, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has 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 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Wagner, R Telephone No. +49 89 2399-7357

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/037471

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☒ on paper

      ☒ in electronic form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☒ furnished subsequently to this Authority for the purposes of search.

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:

   see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2005/037471

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 45,61,62,76-79

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 45,61,62,76-79 (regarding industrial applicability)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/037471

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	25-27,49-56,57-62,64-79
	No:	Claims	1-24,28-48
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-79
Industrial applicability (IA)	Yes:	Claims	1-44,46-60,63-75
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2005/037471

Re Item I
Basis of the report

The sequence listing filed with the letter dated 02.05.06 does not form part of the application as originally filed

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 45,61,62,76-79 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 V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
D1: WO03/105894
D2: WO03/106644

2. Claim 1 is directed to a pharmaceutical composition comprising a monoclonal antibody and a histidine acetate buffer at a pH between 5.5 and 6.5.

The prior art document D1 discloses an antibody formulation at the same pH range (page 21, lines 2-3) in a histidine buffer. The histidine salt used can be acetate (page 20, line 30). Therefore D1 anticipates the subject-matter of independent claim 1. The additional features of dependent claims 2-18 are also disclosed on pates 20 and 21 of D1. The characteristics of the antibody used to limit dependent claims 19-23 are inherent to the antibody used in D1 (see page 4). The additional features of

dependent claims 24, 28-44 are either formulated as a result to be achieved or are inherent to the formulation, or are not considered to be characterising the formulation of claim 1 (vials, tanks etc). Therefore the subject-matter of said claims is not considered to be novel (Article 33(2) PCT).

Claims 25-27 are novel (Article 33(2) PCT) because D1 does not disclose a composition with the precise antibodies of said claims. But the subject-matter of claims 25-27 does not involve an inventive step because it refers to randomly chosen, commercially interesting antibodies and no technical effect can be related to the said choice. Therefore the subject-matter of claims 25-27 does not involve an inventive step (Article 33(3) PCT).

The method of treatment of claim 45 is not new (Article 33(2) PCT) because the antibodies of D1 are also intended for treating a disease.

The combination of features of claim 46 is also disclosed on pages 20 and 21 of D1 and therefore the subject-matter of claim 46 is not novel (Article 33(2) PCT).

In example 1 of D1 (page 57) the physical aggregation of the formulation was measured, with the aim to reduce aggregation. Therefore claims 47, 48, 63 are not new (Article 33(2) PCT).

The pharmaceutical compositions and their medical use of claims 49-56, 57-62, 64-79 differ from the disclosure of D1 only by the random choice of a precise therapeutic antibody. Therefore the subject-matter of said claim is considered to be novel (Article 33(2) PCT) but does not involve an inventive step (Article 33(3) PCT).

Document D2, is a patent application filed by the same applicant as D1 and discloses the same subject-matter relating to the formulation of the antibodies.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

LEE, Wendy, M.
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 02 February 2006 (02.02.2006)	
Applicant's or agent's file reference P2104R1	IMPORTANT NOTIFICATION
International application No. PCT/US2005/037471	International filing date *(day/month/year)* 19 October 2005 (19.10.2005)
International publication date *(day/month/year)* Not yet published	Priority date *(day/month/year)* 20 October 2004 (20.10.2004)
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
20 October 2004 (20.10.2004)	60/620,413	US	03 January 2006 (03.01.2006)

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

FEBRUARY 14, 2006

PTAS

700234361A

700234361A

TIMOTHY R. SCHWARTZ, PH.D.
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 571-272-3350. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, MAIL STOP: ASSIGNMENT SERVICES BRANCH, P.O. BOX 1450, ALEXANDRIA, VA 22313.

RECORDATION DATE: 01/04/2006

REEL/FRAME: 017162/0444
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: P2104R1

ASSIGNOR:
ANDYA, JAMES
DOC DATE: 12/20/2005

ASSIGNOR:
GWEE, SHIANG C.
DOC DATE: 12/20/2005

ASSIGNOR:
LIU, JUN
DOC DATE: 01/04/2006

ASSIGNOR:
SHEN, YU
DOC DATE: 12/20/2005

017162/0444 PAGE 2

ASSIGNEE:
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080-4990

SERIAL NUMBER: 11254182 FILING DATE: 10/19/2005
PATENT NUMBER: ISSUE DATE:
TITLE: ANTIBODY FORMULATIONS

TARA WASHINGTON, EXAMINER
ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

1/04/2006
700234361

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

RECORDATION FORM COVER SHEET
PATENTS ONLY

Commissioner for Patents: Please record the attached original documents or copy thereof.

1. Name of conveying party(ies):

James Andya
Shiang C. Gwee
Jun Liu
Ye Shen

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:

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)
11/254,182

B. Patent No.(s)

Additional numbers attached? _ Yes X No

5. Name and address of party to whom correspondence concerning document should be mailed:

Timothy R. Schwartz, Ph.D.
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

DO NOT USE THIS SPACE

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.

Timothy R. Schwartz, Ph D
Registration No. 52,171

Signature

January 4, 2006
Date

Total number of pages comprising cover sheet, attachments and document: 4

~~425~~
425

Docket No. P2104R1

ASSIGNMENT

WHEREAS, James Andya, a citizen of the U.S., residing at 227 Richmond Drive, Apt. D, Millbrae, CA 94030, and Shiang C. Gwee, a citizen of the U.S., residing at 565 Crespi Drive, Pacifica, CA 94044, and Jun Liu, a citizen of the U.S., residing at 1108 Glacier Avenue, Pacifica, CA 94044, and Ye Shen, a citizen of the U.S., residing at 386 Faxon Avenue, San Francisco, CA 94112, (hereinafter "ASSIGNORS"), have invented a new and useful invention in,

ANTIBODY FORMULATIONS

for which an application Serial No. 11/254,182 (Docket No. P2104R1) for Letters Patent has been by them on October 19, 2005; 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: 12/20/05

James Andya

South San Francisco
Dated: 12/20/05

Shiang C. Gwee

South San Francisco
Dated: 1/4/06

Jun Liu

South San Francisco
Dated: 12/20/05

Ye Shen

P2104R1

PCT REQUEST

Duplicate of Original

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 0002.175)
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	P2104R1
I	Title of invention	ANTIBODY FORMULATIONS
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.	650-225-1000
II-9	Facsimile No.	650-952-9881
III-1	Applicant and/or inventor	
III-1-1	This person is	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	ANDYA, James D.
III-1-5	Address	227 Richmond Drive, Apt. D Millbrae, California 94030 United States of America
III-1-6	State of nationality	US
III-1-7	State of residence	US

PCT REQUEST

Duplicate of Original

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)	GWEE, Shiang C.
III-2-5	Address	565 Crespi Drive Pacifica, California 94044 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)	LIU, Jun
III-3-5	Address	1108 Glacier Avenue Pacifica, California 94044 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)	SHEN, Ye
III-4-5	Address	386 Faxon Avenue San Francisco, California 94112 United States of America
III-4-6	State of nationality	US
III-4-7	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)	LEE, Wendy M.
IV-1-2	Address	GENENTECH, INC. 1 DNA Way South San Francisco, California 94080-4990 United States of America
IV-1-3	Telephone No.	650-225-5416
IV-1-4	Facsimile No.	650-952-9881
IV-1-6	Agent's registration No.	40,378

P2104R1

PCT REQUEST

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IV-2	Additional agent(s)	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	MARSCHANG,Diane L.(35,600); HASAK,Janet E.(28,616); AGARWAL,Atulya R.; ANDRUS,Alex; BARNES,Elizabeth M.; COBURN,Cara M.; CARPENTER,David A.; CONLEY,Deirdre L.; CUI,Steven X.; ELLIOTT,Jennifer L.; EVANS,David W.; JOHNSTON,Sean A.; KALINOWSKI,Grant; KRESNAK,Mark T.; KUBINEC,Jeffrey S.; NAIK,Paul; PLEASURE,Irene; SCHWARTZ,Timothy R.; SHIN,Elinor K.; SVOBODA,Craig G.; TAN,Lee K.; YEUNG,Bonny G.
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	20 October 2004 (20.10.2004)
VI-1-2	Number	60/620,413
VI-1-3	Country	US
VI-2	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
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)

P2104R1

PCT REQUEST

Duplicate of Original

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 purpose 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	76	-
IX-3	Claims	4	-
IX-4	Abstract	1	✓
IX-5	Drawings	34	-
IX-7	TOTAL	120	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	reference no. none	-
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)	LEE, Wendy M.	
X-1-2	Name of signatory		
X-1-3	Capacity		

P2104R1

PCT REQUEST

Original (for SUBMISSION)

VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	5	✓
IX-2	Description	76	-
IX-3	Claims	4	-
IX-4	Abstract	1	✓
IX-5	Drawings	34	-
IX-7	TOTAL	120	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	reference no. none	-
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	Wendy Lee	
X-1-1	Name (LAST, First)	LEE, Wendy M.	
X-1-2	Name of signatory		
X-1-3	Capacity		

P2104R1

PCT REQUEST

Duplicate of Original

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	

433

Attorney Docket P2104R1
Express Mail No.: EV 712 538 913 US
Mailed: October 19, 2005

ANTIBODY FORMULATIONS

This is a non-provisional application filed under 37 CFR 1.53(b) claiming priority to provisional application 60/620,413 filed October 20, 2004, the contents of which are incorporated herein by reference

Field of the Invention

The present invention concerns antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

Background of the Invention

In the past ten years, advances in biotechnology have made it possible to produce a variety of proteins for pharmaceutical applications using recombinant DNA techniques. Because proteins are larger and more complex than traditional organic and inorganic drugs (*i.e.* possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (*i.e.* any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (*i.e.* changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation, deamidation and oxidation. Cleland *et al. Critical Reviews in Therapeutic Drug Carrier Systems* 10(4): 307-377 (1993).

Antibody Formulations

Included in the proteins used for pharmaceutical applications are antibodies. An example of an antibody useful for therapy is an antibody which binds to the HER2 antigen, such as Pertuzumab.

US Patent No. 6,339,142 describes a HER2 antibody composition comprising a mixture of anti-HER2 antibody and one or more acidic variants thereof, wherein the amount of the acidic variant(s) is less than about 25%. Trastuzumab is an exemplified HER2 antibody.

US Patent Nos. 6,267,958 and 6,685,940 (Andya *et al.*) describe lyophilized antibody formulations, including HER2 and IgE antibody formulations. WO97/04807 and US 2004/0197326A1 (Fick *et al.*) describe methods for treating allergic asthma with an IgE antibody. WO99/01556 (Lowman *et al.*) relates to IgE antibody with aspartyl residues prone to isomerization, and improved variants thereof. US 2002/0045571 (Liu *et al.*) provides reduced viscosity concentrated protein formulations, exemplified by humanized IgE antibody formulations, rhuMAb E25 and E26. WO 02/096457 and US 2004/0170623 (Arvinte *et al.*) describes stable liquid formulations comprising anti-IgE antibody E25. See, also, US 2004/0197324 A1 (Liu and Shire) concerning high concentration anti-IgE formulation.

US Patent No. 6,171,586 (Lam *et al.*) describes stable aqueous antibody formulations. A F(ab')2 rhuMAb CD18 antibody was formulated in sodium acetate and histidine-HCl buffers. The preferred formulation for rhuMAb CD18 was 10mM sodium acetate, 8% trehalose, 0.01% TWEEN 20™, pH 5.0. Acetate (pH 5.0) formulations of rhuMAb CD20 stored at 40°C for one month demonstrated greater stability than those samples formulated in histidine (pH 5.0 or 6.0).

US 2003/0190316 (Kakuta *et al.*) concerns formulated antibody hPM-1, a humanized IL-6 receptor antibody. Monomer loss was the greatest in sodium citrate (pH 6.7), followed by sodium phosphate (pH 6.8), Tris-HCl (pH 7.2), histidine-HCl (pH 7.2) and glycine (pH 7.6) in descending order. The effect of phosphate-Na (pH 6.5), phosphate-His (pH 6.0 or 6.5), His-HCl (pH 6.5), and phosphate-Na (pH 6.0) on the stability of hPM-1 was assessed.

WO2004/071439 (Burke *et al.*) state that impurities arose in a natalizumab (anti-alpha4 integrin humanized monoclonal antibody) formulation from the degradation of polysorbate 80, apparently through an oxidation reaction involving metal ions and hisitidine. Thus, a phosphate buffer was selected.

WO 2000/066160 (English language counterpart EP 1 174 148A1) (Okada *et al.*) refers to a formulation of a humanized C4G1 antibody which binds to a fibrinogen receptor of a human platelet membrane glycoprotein GPIIb/IIIa, in a sodium phosphate or sodium citrate buffer.

WO2004/019861 (Johnson *et al.*) concerns CDP870, a pegylated anti-TNFα Fab fragment, formulated at 200mg/ml in 50mM sodium acetate (pH 5.5) and 125mM sodium chloride.

WO2004/004639 (Nesta, P.) refers to a formulation for huC242-DM1, a tumor-activated immunotoxin, in a 50mM succinic acid buffer (pH 6.0) and sucrose (5%w/v).

WO03/039485 (Kaisheva *et al.*) found that Daclizumab (a humanized IL-2 receptor antibody) had the highest stability in sodium succinate buffer at pH 6.0, and rapidly lost potency in histidine as the buffer oxidized.

WO 2004/001007 concerns a CD80 monoclonal antibody in a histidine HCl, sodium acetate or sodium citrate buffer.

US Patent No. 6,252,055 (Relton, J.) refers to anti-CD4 and anti-CD23 antibodies formulated in maleate, succinate, sodium acetate or phosphate buffers, with phosphate being identified as the preferred buffer.

US Patent No. 5,608,038 (Eibl *et al.*) refers to highly concentrated polyclonal immunoglobulin preparations with immunoglobulin, glucose or sucrose, and sodium chloride therein.

WO03/015894 (Oliver *et al.*) refers to an aqueous formulation of 100mg/mL SYNAGIS®, 25mM histidine-HCl, 1.6mM glycine, pH 6.0, and a lyophilized SYNAGIS® which when formulated (before lyophilization) contains 25mM histidine, 1.6mM glycine and 3% w/v mannitol at pH 6.0.

US 2004/0191243 A1 (Chen *et al.*) reports formulation of ABX-IL8, a human IgG2 antibody.

US 2003/0113316 A1 (Kaisheva *et al.*) refers to a lyophilized anti-IL2 receptor antibody formulation.

HER2 Antibodies

The HER family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, or HER1), HER2 (ErbB2 or $p185^{neu}$), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

EGFR, encoded by the *erb*B1 gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF-α), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway. Baselga and Mendelsohn *Pharmac. Ther.* 64:127-154 (1994). Monoclonal antibodies directed against the EGFR or its ligands, TGF-α and EGF, have been evaluated as therapeutic agents in the treatment of such malignancies. See, *e.g.*, Baselga and Mendelsohn., *supra*; Masui *et al. Cancer Research* 44:1002-1007 (1984); and Wu *et al. J. Clin. Invest.* 95:1897-1905 (1995).

The second member of the HER family, $p185^{neu}$, was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the *neu* proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homolog of *neu* is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon *et al.*, *Science*, 235:177-182 (1987); Slamon *et al.*, *Science*, 244:707-712 (1989); and US Pat No. 4,968,603). To date, no point mutation analogous to that in the *neu* proto-oncogene has been reported for human tumors. Overexpression of HER2 (frequently but not uniformly due to gene amplification) has also been observed in other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. See, among others, King *et al.*, *Science*, 229:974 (1985); Yokota *et al.*, *Lancet*: 1:765-767 (1986); Fukushige *et al.*, *Mol Cell Biol.*, 6:955-958 (1986); Guerin *et al.*, *Oncogene Res.*, 3:21-31 (1988); Cohen *et al.*, *Oncogene*, 4:81-88 (1989); Yonemura *et al.*, *Cancer Res.*, 51:1034 (1991); Borst *et al.*, *Gynecol. Oncol.*, 38:364 (1990); Weiner *et al.*, *Cancer Res.*, 50:421-425 (1990); Kern *et al.*, *Cancer Res.*, 50:5184 (1990); Park *et al.*, *Cancer Res.*, 49:6605 (1989); Zhau *et al.*, *Mol. Carcinog.*, 3:254-257 (1990); Aasland *et al. Br. J. Cancer* 57:358-363 (1988); Williams *et al. Pathobiology* 59:46-52 (1991); and McCann *et al.*, *Cancer*, 65:88-92 (1990). HER2 may be overexpressed in prostate cancer (Gu *et al. Cancer Lett.* 99:185-9 (1996); Ross *et al. Hum. Pathol.* 28:827-33 (1997); Ross *et al. Cancer* 79:2162-70 (1997); and Sadasivan *et al. J. Urol.* 150:126-31 (1993)).

Antibodies directed against the rat $p185^{neu}$ and human HER2 protein products have been described. Drebin and colleagues have raised antibodies against the rat *neu* gene product, $p185^{neu}$. See, for example, Drebin *et al.*, *Cell* 41:695-706 (1985); Myers *et al.*, *Meth. Enzym.* 198:277-290 (1991); and WO94/22478. Drebin *et al. Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions of $p185^{neu}$ result

in synergistic anti-tumor effects on *neu*-transformed NIH-3T3 cells implanted into nude mice. See also U.S. Patent 5,824,311 issued October 20, 1998.

Hudziak *et al.*, *Mol. Cell. Biol.* 9(3):1165-1172 (1989) describe the generation of a panel of HER2 antibodies which were characterized using the human breast tumor cell line SK-BR-3. Relative cell proliferation of the SK-BR-3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, 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 HER2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also U.S. Patent No. 5,677,171 issued October 14, 1997. The HER2 antibodies discussed in Hudziak *et al.* are further characterized in Fendly *et al. Cancer Research* 50:1550-1558 (1990); Kotts *et al. In Vitro* 26(3):59A (1990); Sarup *et al. Growth Regulation* 1:72-82 (1991); Shepard *et al. J. Clin. Immunol.* 11(3):117-127 (1991); Kumar *et al. Mol. Cell. Biol.* 11(2):979-986 (1991); Lewis *et al. Cancer Immunol. Immunother.* 37:255-263 (1993); Pietras *et al. Oncogene* 9:1829-1838 (1994); Vitetta *et al. Cancer Research* 54:5301-5309 (1994); Sliwkowski *et al. J. Biol. Chem.* 269(20):14661-14665 (1994); Scott *et al. J. Biol. Chem.* 266:14300-5 (1991); D'souza *et al. Proc. Natl. Acad. Sci.* 91:7202-7206 (1994); Lewis *et al. Cancer Research* 56:1457-1465 (1996); and Schaefer *et al. Oncogene* 15:1385-1394 (1997).

A recombinant humanized version of the murine HER2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2, Trastuzumab or HERCEPTIN™; U.S. Patent No. 5,821,337) is clinically active in patients with HER2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 (1996)). Trastuzumab received marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

Other HER2 antibodies with various properties have been described in Tagliabue *et al. Int. J. Cancer* 47:933-937 (1991); McKenzie *et al. Oncogene* 4:543-548 (1989); Maier *et al. Cancer Res.* 51:5361-5369 (1991); Bacus *et al. Molecular Carcinogenesis* 3:350-362 (1990); Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991); Bacus *et al. Cancer Research* 52:2580-2589 (1992); Xu *et al. Int. J. Cancer* 53:401-408 (1993); WO94/00136; Kasprzyk *et al. Cancer Research* 52:2771-2776 (1992);Hancock *et al. Cancer Res.* 51:4575-4580 (1991); Shawver *et al. Cancer Res.* 54:1367-1373 (1994); Arteaga *et al. Cancer Res.* 54:3758-3765 (1994); Harwerth *et al. J. Biol. Chem.* 267:15160-15167 (1992); U.S. Patent No. 5,783,186; and Klapper *et al. Oncogene* 14:2099-2109 (1997).

Homology screening has resulted in the identification of two other HER receptor family members; HER3 (US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989)) and HER4 (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)). Both of these receptors display increased expression on at least some breast cancer cell lines.

The HER receptors are generally found in various combinations in cells and heterodimerization is thought to increase the diversity of cellular responses to a variety of HER ligands (Earp *et al. Breast Cancer Research and Treatment* 35: 115-132 (1995)). EGFR is bound by six different ligands; epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin, heparin binding epidermal growth factor (HB-EGF), betacellulin and epiregulin (Groenen *et al. Growth Factors* 11:235-257 (1994)). A family of heregulin proteins resulting from alternative splicing of a single gene are ligands for HER3 and HER4. The heregulin family includes alpha, beta and gamma heregulins (Holmes *et al.*, *Science*, 256:1205-1210 (1992); U.S. Patent No. 5,641,869; and Schaefer *et al. Oncogene* 15:1385-1394 (1997)); neu differentiation factors (NDFs), glial growth factors (GGFs); acetylcholine receptor inducing activity (ARIA); and sensory and motor neuron derived factor (SMDF). For a review, see Groenen *et al. Growth Factors* 11:235-257 (1994); Lemke, G. *Molec. & Cell. Neurosci.* 7:247-262 (1996) and Lee *et al. Pharm. Rev.* 47:51-85 (1995). Recently three additional HER ligands were identified; neuregulin-2 (NRG-2) which is reported to bind either HER3 or HER4 (Chang *et al. Nature* 387 509-512 (1997); and Carraway *et al Nature* 387:512-516 (1997)); neuregulin-3 which binds HER4 (Zhang *et al. PNAS* (USA) 94(18):9562-7 (1997)); and neuregulin-4 which binds HER4 (Harari *et al. Oncogene* 18:2681-89 (1999)) HB-EGF, betacellulin and epiregulin also bind to HER4.

While EGF and TGFα do not bind HER2, EGF stimulates EGFR and HER2 to form a heterodimer, which activates EGFR and results in transphosphorylation of HER2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the HER2 tyrosine kinase. See Earp *et al.*, *supra*. Likewise, when HER3 is co-expressed with HER2, an active signaling complex is formed and antibodies directed against HER2 are capable of disrupting this complex (Sliwkowski *et al.*, *J. Biol. Chem.*, 269(20):14661-14665 (1994)). Additionally, the affinity of HER3 for heregulin (HRG) is increased to a higher affinity state when co-expressed

with HER2. See also, Levi *et al.*, *Journal of Neuroscience* 15: 1329-1340 (1995); Morrissey *et al.*, *Proc. Natl. Acad. Sci. USA* 92: 1431-1435 (1995); and Lewis *et al.*, *Cancer Res.*, 56:1457-1465 (1996) with respect to the HER2-HER3 protein complex. HER4, like HER3, forms an active signaling complex with HER2 (Carraway and Cantley, *Cell* 78:5-8 (1994)).

To target the HER signaling pathway, rhuMAb 2C4 (Pertuzumab, OMNITARG™) was developed as a humanized antibody that inhibits the dimerization of HER2 with other HER receptors, thereby inhibiting ligand-driven phosphorylation and activation, and downstream activation of the RAS and AKT pathways. In a phase I trial of Pertuzumab as a single agent for treating solid tumors, 3 subjects with advanced ovarian cancer were treated with Pertuzumab. One had a durable partial response, and an additional subject had stable disease for 15 weeks Agus *et al. Proc Am Soc Clin Oncol* 22: 192, Abstract 771 (2003).

DR5 Antibodies

Various ligands and receptors belonging to the tumor necrosis factor (TNF) superfamily have been identified in the art. Included among such ligands are tumor necrosis factor-alpha ("TNF-alpha"), tumor necrosis factor-beta ("TNF-beta" or "lymphotoxin-alpha"), lymphotoxin-beta ("LT-beta"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, LIGHT, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as Apo2L or TRAIL), Apo-3 ligand (also referred to as TWEAK), APRIL, OPG ligand (also referred to as RANK ligand, ODF, or TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK) (See, *e.g.*, Ashkenazi, *Nature Review*, 2:420-430 (2002); Ashkenazi and Dixit, *Science*, 281:1305-1308 (1998); Ashkenazi and Dixit, *Curr. Opin. Cell Biol.*, 11:255-260 (2000); Golstein, *Curr. Biol.*, 7:750-753 (1997) Wallach, *Cytokine Reference*, Academic Press, 2000, pages 377-411; Locksley *et al.*, *Cell*, 104:487-501 (2001); Gruss and Dower, *Blood*, 85:3378-3404 (1995); Schmid *et al.*, *Proc. Natl. Acad. Sci.*, 83:1881 (1986); Dealtry *et al.*, *Eur. J. Immunol.*, 17:689 (1987); Pitti *et al.*, *J. Biol. Chem.*, 271:12687-12690 (1996); Wiley *et al.*, *Immunity*, 3:673-682 (1995); *Browning et al.*, *Cell*, 72:847-856 (1993); Armitage *et al. Nature*, 357:80-82 (1992), WO 97/01633 published January 16, 1997; WO 97/25428 published July 17, 1997; Marsters *et al.*, *Curr. Biol.*, 8:525-528 (1998); Chicheportiche *et al.*, *Biol. Chem.*, 272:32401-32410 (1997); Hahne *et al.*, *J. Exp. Med.*, 188:1185-1190 (1998); WO98/28426 published July 2, 1998; WO98/46751 published October 22, 1998; WO98/18921 published May 7, 1998; Moore *et al.*, *Science*, 285:260-263 (1999); Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); Schneider *et al.*, *J. Exp. Med.*, 189:1747-1756 (1999); Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274:15978-15981 (1999)).

Induction of various cellular responses mediated by such TNF family ligands is typically initiated by their binding to specific cell receptors. Some, but not all, TNF family ligands bind to, and induce various biological activity through, cell surface "death receptors" to activate caspases, or enzymes that carry out the cell death or apoptosis pathway (Salvesen *et al.*, *Cell*, 91:443-446 (1997)). Included among the members of the TNF receptor superfamily identified to date are TNFR1, TNFR2, TACI, GITR,, CD27, OX-40, CD30, CD40, HVEM, Fas (also referred to as Apo-1 or CD95), DR4 (also referred to as TRAIL-R1), DR5 (also referred to as Apo-2 or TRAIL-R2), DcR1, DcR2, osteoprotegerin (OPG), RANK and Apo-3 (also referred to as DR3 or TRAMP) .

Most of these TNF receptor family members share the typical structure of cell surface receptors including extracellular, transmembrane and intracellular regions, while others are found naturally as soluble proteins lacking a transmembrane and intracellular domain. The extracellular portion of typical TNFRs contains

a repetitive amino acid sequence pattern of multiple cysteine-rich domains (CRDs), starting from the NH_2-terminus.

The ligand referred to as Apo-2L or TRAIL was identified several years ago as a member of the TNF family of cytokines. (see, *e.g.*, Wiley *et al.*, *Immunity*, 3:673-682 (1995); Pitti *et al.*, *J. Biol. Chem.*, 271:12697-12690 (1996); WO 97/01633; WO 97/25428; US Patent 5,763,223 issued June 9, 1998; US Patent 6,284,236 issued September 4, 2001). The full-length native sequence human Apo2L/TRAIL polypeptide is a 281 amino acid long, Type II transmembrane protein. Some cells can produce a natural soluble form of the polypeptide, through enzymatic cleavage of the polypeptide's extracellular region (Mariani *et al.*, *J. Cell. Biol.*, 137:221-229 (1997)). Crystallographic studies of soluble forms of Apo2L/TRAIL reveal a homotrimeric structure similar to the structures of TNF and other related proteins (Hymowitz *et al.*, *Molec. Cell*, 4:563-571 (1999); Cha *et al.*, *Immunity*, 11:253-261 (1999); Mongkolsapaya *et al.*, *Nature Structural Biology*, 6:1048 (1999); Hymowitz *et al.*, *Biochemistry*, 39:633-644 (2000)). Apo2L/TRAIL, unlike other TNF family members however, was found to have a unique structural feature in that three cysteine residues (at position 230 of each subunit in the homotrimer) together coordinate a zinc atom, and that the zinc binding is important for trimer stability and biological activity. (Hymowitz *et al.*, *supra*; Bodmer *et al.*, *J. Biol. Chem.*, 275:20632-20637 (2000)).

It has been reported in the literature that Apo2L/TRAIL may play a role in immune system modulation, including autoimmune diseases such as rheumatoid arthritis (see, *e.g.*, Thomas *et al.*, *J. Immunol.*, 161:2195-2200 (1998); Johnsen *et al.*, *Cytokine*, 11:664-672 (1999); Griffith *et al.*, *J. Exp. Med.*, 189:1343-1353 (1999); Song *et al.*, *J. Exp. Med.*, 191:1095-1103 (2000)).

Soluble forms of Apo2L/TRAIL have also been reported to induce apoptosis in a variety of cancer cells, including colon, lung, breast, prostate, bladder, kidney, ovarian and brain tumors, as well as melanoma, leukemia, and multiple myeloma (see, *e.g.*, Wiley *et al.*, *supra*; Pitti *et al.*, *supra*; US Patent 6,030,945 issued February 29, 2000; US Patent 6,746,668 issued June 8, 2004; Rieger *et al.*, *FEBS Letters*, 427:124-128 (1998); Ashkenazi *et al.*, *J. Clin. Invest.*, 104:155-162 (1999); Walczak *et al.*, *Nature Med.*, 5:157-163 (1999); Keane *et al.*, *Cancer Research*, 59:734-741 (1999); Mizutani *et al.*, *Clin. Cancer Res.*, 5:2605-2612 (1999); Gazitt, *Leukemia*, 13:1817-1824 (1999); Yu *et al.*, *Cancer Res.*, 60:2384-2389 (2000); Chinnaiyan *et al.*, *Proc. Natl. Acad. Sci.*, 97:1754-1759 (2000)). *In vivo* studies in murine tumor models further suggest that Apo2L/TRAIL, alone or in combination with chemotherapy or radiation therapy, can exert substantial anti-tumor effects (see, *e.g.*, Ashkenazi *et al.*, *supra*; Walzcak *et al.*, *supra*; Gliniak *et al.*, *Cancer Res.*, 59:6153-6158 (1999); Chinnaiyan *et al.*, *supra*; Roth *et al.*, *Biochem. Biophys. Res. Comm.*, 265:1999 (1999); PCT Application US/00/15512; PCT Application US/01/23691). In contrast to many types of cancer cells, most normal human cell types appear to be resistant to apoptosis induction by certain recombinant forms of Apo2L/TRAIL (Ashkenazi *et al.*, *supra*; Walzcak *et al.*, *supra*). Jo *et al.* has reported that a polyhistidine-tagged soluble form of Apo2L/TRAIL induced apoptosis *in vitro* in normal isolated human, but not non-human, hepatocytes (Jo *et al.*, *Nature Med.*, 6:564-567 (2000); see also, Nagata, *Nature Med.*, 6:502-503 (2000)). It is believed that certain recombinant Apo2L/TRAIL preparations may vary in terms of biochemical properties and biological activities on diseased versus normal cells, depending, for example, on the presence or absence of a tag molecule, zinc content, and % trimer content (See, Lawrence *et al.*, *Nature Med.*, Letter to the Editor, 7:383-385 (2001); Qin *et al.*, *Nature Med.*, Letter to the Editor, 7:385-386 (2001)).

Apo2L/TRAIL has been found to bind at least five different receptors. At least two of the receptors which bind Apo2L/TRAIL contain a functional, cytoplasmic death domain. One such receptor has been referred to as "DR4" (and alternatively as TR4 or TRAIL-R1) (Pan *et al.*, *Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002).

Another such receptor for Apo2L/TRAIL has been referred to as DR5 (it has also been alternatively referred to as Apo-2; TRAIL-R or TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER) (see, *e.g.*, Sheridan *et al.*, *Science*, 277:818-821 (1997), Pan *et al.*, *Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton et al., Curr. Biol., 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; IS 6,743,625 issued June 1, 2004). Like DR4, DR5 is reported to contain a cytoplasmic death domain and be capable of signaling apoptosis upon ligand binding (or upon binding a molecule, such as an agonist antibody, which mimics the activity of the ligand). The crystal structure of the complex formed between Apo-2L/TRAIL and DR5 is described in Hymowitz *et al.*, *Molecular Cell*, 4:563-571 (1999).

Upon ligand binding, both DR4 and DR5 can trigger apoptosis independently by recruiting and activating the apoptosis initiator, caspase-8, through the death-domain-containing adaptor molecule referred to as FADD/Mort1 (Kischkel *et al.*, *Immunity*, 12:611-620 (2000); Sprick *et al.*, *Immunity*, 12:599-609 (2000); Bodmer *et al.*, *Nature Cell Biol.*, 2:241-243 (2000)).

Apo2L/TRAIL has been reported to also bind those receptors referred to as DcR1, DcR2 and OPG, which believed to function as inhibitors, rather than transducers of signaling (see., *e.g.*, DcR1 (also referred to as TRID, LIT or TRAIL-R3) (Pan *et al.*, *Science*, 276:111-113 (1997); Sheridan *et al.*, *Science*, 277:818-821 (1997); McFarlane *et al.*, *J. Biol. Chem.*, 272:25417-25420 (1997); Schneider *et al.*, *FEBS Letters*, 416:329-334 (1997); Degli-Esposti *et al.*, *J. Exp. Med.*, 186:1165-1170 (1997); and Mongkolsapaya *et al.*, *J. Immunol.*, 160:3-6 (1998)); DcR2 (also called TRUNDD or TRAIL-R4) (Marsters *et al.*, *Curr. Biol.*, 7:1003-1006 (1997); Pan *et al.*, *FEBS Letters*, 424:41-45 (1998); Degli-Esposti *et al.*, *Immunity*, 7:813-820 (1997)), and OPG. In contrast to DR4 and DR5, the DcR1 and DcR2 receptors do not signal apoptosis.

Certain antibodies which bind to the DR4 and/or DR5 receptors have been reported in the literature. For example, anti-DR4 antibodies directed to the DR4 receptor and having agonistic or apoptotic activity in certain mammalian cells are described in, *e.g.*, WO 99/37684 published July 29, 1999; WO 00/73349 published July 12, 2000; WO 03/066661 published August 14, 2003. See, also, *e.g.*, Griffith *et al.*, *J. Immunol.*, 162:2597-2605 (1999); Chuntharapai *et al.*, *J. Immunol.*, 166:4891-4898 (2001); WO 02/097033 published December 2, 2002; WO 03/042367 published May 22, 2003; WO 03/038043 published May 8, 2003; WO 03/037913 published

May 8, 2003. Certain anti-DR5 antibodies have likewise been described. see, *e.g.*, WO 98/51793 published November 8, 1998; Griffith *et al., J. Immunol.*, 162:2597-2605 (1999); Ichikawa *et al., Nature Med.*, 7:954-960 (2001); Hylander *et al.*, "An Antibody to DR5 (TRAIL-Receptor 2) Suppresses the Growth of Patient Derived Gastrointestinal Tumors Grown in SCID mice", Abstract, 2d International Congress on Monoclonal Antibodies in Cancers, Aug. 29-Sept. 1, 2002, Banff, Alberta, Canada; WO 03/038043 published May 8, 2003; WO 03/037913 published May 8, 2003. In addition, certain antibodies having cross-reactivity to both DR4 and DR5 receptors have been described (see, *e.g.*, US patent 6,252,050 issued June 26, 2001).

Summary of the Invention

The invention herein relates, at least in part, to the identification of histidine-acetate, pH 5.5 to 6.5, as a particularly useful buffer for formulating monoclonal antibodies, especially full length IgG1 antibodies which are susceptible to deamidation and/or aggregation. The formulation retards degradation of the antibody product therein.

Thus, in a first aspect, the invention concerns a stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5. The monoclonal antibody preferably binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

In addition, the invention concerns a method of treating a disease or disorder in a subject comprising administering the formulation to a subject in an amount effective to treat the disease or disorder.

In another aspect, the invention concerns a pharmaceutical formulation comprising: (a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL: (b) histidine-acetate buffer, pH 5.5 to 6.5; (c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and (d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

The invention also provides a method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5.

In yet a further aspect, the invention concerns a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant.

The invention also relates to a pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The invention also pertains to a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

In another aspect, the invention concerns a pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

In yet another aspect, the invention provides a method of treating cancer in a subject, comprising administering the pharmaceutical formulation to the subject in an amount effective to treat the cancer.

The invention also concerns a vial with a stopper pierceable by a syringe or a stainless steel tank comprising the formulation inside the vial or tank, optionally in frozen form.

Moreover, the invention provides a method of making a pharmaceutical formulation comprising: (a) preparing the monoclonal antibody formulation; and (b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

Brief Description of the Drawings

Figure 1 depicts Domains I-IV (SEQ ID Nos.19-22, respectively) of the extracellular domain of HER2.

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (V_L) (Fig. 2A) and variable heavy (V_H) (Fig. 2B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 1 and 2, respectively); V_L and V_H domains of humanized 2C4 version 574 (SEQ ID Nos. 3 and 4, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 5 and 6, respectively). Asterisks identify differences between humanized 2C4 version 574 and murine monoclonal antibody 2C4 or between humanized 2C4 version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

Figures 3A and 3B show the amino acid sequences of Pertuzumab light chain and heavy chain (SEQ ID Nos. 15 and 16, respectively). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figures 4A and 4B show the amino acid sequences of Pertuzumab light and heavy chain, each including an intact amino terminal signal peptide sequence (SEQ ID Nos. 17 and 18, respectively).

Figure 5 depicts, schematically, binding of 2C4 at the heterodimeric binding site of HER2, thereby preventing heterodimerization with activated EGFR or HER3.

Figure 6 depicts coupling of HER2/HER3 to the MAPK and Akt pathways.

Figure 7 compares activities of Trastuzumab and Pertuzumab.

Figure 8 depicts stability of Pertuzumab formulation by ion exchange (IEX) analyses.

Figure 9 shows stability of Pertuzumab formulation by size exclusion chromatography (SEC) analysis.

Figure 10 reflects physical stability Pertuzumab in different formulations.

Figure 11 is from an agitation study of Pertuzumab liquid formulations.

Figure 12 is from another agitation study of Pertuzumab liquid formulations.

Figure 13 is from a freeze-thawing study of Pertuzumab formulation.

Figures 14A and 14B show the amino acid sequences of Trastuzumab light chain (SEQ ID No. 13) and heavy chain (SEQ ID No. 14).

Figures 15A and 15B depict a variant Pertuzumab light chain sequence (SEQ ID No. 23) and a variant Pertuzumab heavy chain sequence (SEQ ID No. 24).

Figure 16A and 16B shows oligosaccharide structures commonly observed in IgG antibodies.

Figures 17A and 17B show the sequences of the light and heavy chains (SEQ ID Nos. 37-44) of specific anti-IgE antibodies E25, E26, HAE1 and Hu-901. In Fig. 17A, the variable light domain ends with the residues VEIK, residue 111. In Fig. 17B, the variable heavy domain ends with the residues VTVSS, around residue 120.

Figure 18A is a sequence alignment comparing the amino acid sequences of the variable light domain (V_L) of each of murine 2H7 (SEQ ID No. 25), humanized 2H7v16 variant (SEQ ID No. 26), and the human

kappa light chain subgroup I (SEQ ID No. 27). The CDRs of V_L of 2H7 and hu2H7v16 are as follows: CDR1 (SEQ ID No. 57). CDR2 (SEQ ID No. 58). and CDR3 (SEQ ID No. 59).

Figure 18B is a sequence alignment comparing the amino acid sequences of the variable heavy domain (V_H) of each of murine 2H7 (SEQ ID No. 28), humanized 2H7v16 variant (SEQ ID No. 29), and the human consensus sequence of the heavy chain subgroup III (SEQ ID No. 30). The CDRs of V_H of 2H7 and hu2H7v16 are as follows: CDR1 (SEQ ID No. 60), CDR2 (SEQ ID No. 61), and CDR3 (SEQ ID No. 62).

In Fig. 18A and Fig. 18B, the CDR1, CDR2 and CDR3 in each chain are enclosed within brackets, flanked by the framework regions, FR1-FR4, as indicated. 2H7 refers to murine 2H7 antibody. The asterisks in between two rows of sequences indicate the positions that are different between the two sequences. Residue numbering is according to Kabat *et al. Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), with insertions shown as a, b, c, d, and e.

Figure 19 depicts variable domain sequences of three different VEGF antibodies with SEQ ID Nos. 31-36.

Figure 20 shows size exclusion chromatography (SEC) elution profile of the following Apomab samples: (a) control and formulations prepared at (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 and (e) pH 7.0. The formulated samples were stored at 40°C for 2 months prior to the analysis.

Figure 21 depicts pH rate profile for the loss in Apomab antibody monomer during storage. Monomer kinetics by SEC was monitored during storage at 30°C and 40°C and the first-order rate constants were calculated.

Figure 22 provides ion exchange chromatography (IEC) elution profile of Apomab samples as follows: (a) control and formulations prepared at (b) pH 4.0, (c) pH 5.0, (d) pH 6.0 and (e) pH 7.0. The formulated samples were stored at 40°C for 2 months prior to the analysis.

Figure 23 shows pH rate profile for the loss in IEC main peak during storage. Main peak kinetics by IEC was monitored during storage at 30°C and 40°C and the first-order rate constants were calculated.

Figure 24 shows the nucleotide sequence of human Apo-2 ligand cDNA (SEQ ID No. 45) and its derived amino acid sequence (SEQ ID No. 46). The "N" at nucleotide position 447 (in SEQ ID No. 45) is used to indicate the nucleotide base may be a "T" or "G".

Figures 25A and 25B show the 411 amino acid sequence of human DR5 receptor (SEQ ID No. 47) as published in WO 98/51793 on November 19, 1998, and the encoding nucleotide sequence (SEQ ID No. 48).

Figures 26A and 26B show the 440 amino acid sequence of human DR5 receptor (SEQ ID No. 49) and the encoding nucleotide sequence (SEQ ID No. 50), as also published in WO 98/35986 on August 20, 1998.

Figure 27 shows the Apomab 7.3 heavy chain amino acid sequence (SEQ ID No. 51).

Figure 28 shows the Apomab 7.3 light chain amino acid sequence (SEQ ID No.52).

Figures 29 show the alignment of 16E2 heavy chain (SEQ ID No. 53) and Apomab 7.3 heavy chain (SEQ ID No. 51) amino acid sequences.

Figure 30 shows the alignment of 16E2 light chain (SEQ ID No. 54) and Apomab 7.3 light chain (SEQ ID No. 52) amino acid sequences.

Figures 31A and 31B depict the variable heavy amino acid sequence (Fig. 31A; SEQ ID No. 55) and variable light amino acid sequence (Fig. 31B; SEQ ID No. 56) of Apomab 7.3. CDR residues are identified in bold.

Figure 32 shows an alignment of the mature 2H7v16 and 2H7v511 light chains (SEQ ID Nos. 63 and 64, respectively). Sequences shown with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Figure 33 shows an alignment of the the mature 2H7v16 and 2H7v511 heavy chains (SEQ ID Nos. 65 and 66, respectively). Sequences shown with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Detailed Description of the Preferred Embodiments

I. Definitions

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

Herein, a "frozen" formulation is one at a temperature below 0°C. Generally, the frozen formulation is not freeze-dried, nor is it subjected to prior, or subsequent, lyophilization. Preferably, the frozen formulation comprises frozen drug substance for storage (in stainless steel tank) or frozen drug product (in final vial configuration).

A "stable" formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Preferably, the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993), for example. Stability can be measured at a selected temperature for a selected time period. Preferably, the formulation is stable at about 40°C for at least about 2-4 weeks, and/or stable at about 5°C and/or 15°C for at least 3 months, and/or stable at about -20°C for at least 3 months or at least 1 year. Furthermore, the formulation is preferably stable following freezing (to, *e.g.*, -70°C) and thawing of the formulation, for example following 1, 2 or 3 cycles of freezing and thawing. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc. Instability may involve any one or more of: aggregation, deamidation (*e.g.* Asn deamidation), oxidation (*e.g.* Met oxidation), isomerization (*e.g.* Asp isomerization), clipping/hydrolysis/fragmentation (*e.g.* hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc.

A "deamidated" monoclonal antibody herein is one in which one or more asparagine residue thereof has been derivitized, *e.g.* to an aspartic acid or an iso-aspartic acid.

An antibody which is "susceptible to deamidation" is one comprising one or more residue which has been found to be prone to deamidate.

An antibody which is "susceptible to aggregation" is one which has been found to aggregate with other antibody molecule(s), especially upon freezing and/or agitation.

An antibody which is "susceptible to fragmentation" is one which has been found to be cleaved into two or more fragments, for example at a hinge region thereof.

By "reducing deamidation, aggregation, or fragmentation" is intended preventing or decreasing the amount of deamidation, aggregation, or fragmentation relative to the monoclonal antibody formulated at a different pH or in a different buffer.

Herein, "biological activity" of a monoclonal antibody refers to the ability of the antibody to bind to antigen and result in a measurable biological response which can be measured *in vitro* or *in vivo*. Such activity may be antagonistic (for example where the antibody is a HER2 antibody) or agonistic (for instance where the antibody binds DR5). In the case of Pertuzumab, in one embodiment, the biological activity refers to the ability of the formulated antibody to inhibit proliferation of the human breast cancer cell line MDA-MB-175-VII. Where the antibody is Apomab, the biological activity can refer, for example, to the ability of the formulated antibody to kill colon carcinoma, Colo205, cells.

By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffer of this invention preferably has a pH in the range from about 5.0 to about 7.0, preferably from about 5.5 to about 6.5, for example from about 5.8 to about 6.2, and most preferably has a pH of about 6.0. Examples of buffers that will control the pH in this range include acetate, succinate, succinate, gluconate, histidine, citrate, glycylglycine and other organic acid buffers. The preferred buffer herein is a histidine buffer.

A "histidine buffer" is a buffer comprising histidine ions. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, histidine sulfate. The preferred histidine buffer identified in the examples herein was found to be histidine acetate. In the preferred embodiment, the histidine acetate buffer is prepared by titrating L-histidine (free base, solid) with acetic acid (liquid). Preferably, the histidine buffer or histidine-acetate buffer is at pH 5.5 to 6.5, preferably pH 5.8 to 6.2.

A "saccharide" herein comprises the general composition (CH2O)n and derivatives thereof, including monosaccharides, disaccharides, trisaccharides, polysaccharides, sugar alcohols, reducing sugars, nonreducing sugars, etc. Examples of saccharides herein include glucose, sucrose, trehalose, lactose, fructose, maltose, dextran, glycerin, dextran, erythritol, glycerol, arabitol, sylitol, sorbitol, mannitol, mellibiose, melezitose, raffinose, mannotriose, stachyose, maltose, lactulose, maltulose, glucitol, maltitol, lactitol, iso-maltulose, etc. The preferred saccharide herein is a nonreducing disaccharide, such as trehalose or sucrose.

Herein, a "surfactant" refers to a surface-active agent, preferably a nonionic surfactant. Examples of surfactants herein include polysorbate (for example, polysorbate 20 and, polysorbate 80); poloxamer (*e.g.*

poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (*e.g.* lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT™ series (Mona Industries, Inc., Paterson, New Jersey); polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (*e.g.* Pluronics, PF68 etc); etc. The preferred surfactant herein is polysorbate 20.

A "HER receptor" is a receptor protein tyrosine kinase which belongs to the HER receptor family and includes EGFR, HER2, HER3 and HER4 receptors and other members of this family to be identified in the future. The HER receptor will generally comprise an extracellular domain, which may bind an HER 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. Preferably the HER receptor is native sequence human HER receptor.

The extracellular domain of HER2 comprises four domains, Domain I (amino acid residues from about 1-195), Domain II (amino acid residues from about 196-320), Domain III (amino acid residues from about 321-488), and Domain IV (amino acid residues from about 489-632) (residue numbering without signal peptide). See Garrett *et al. Mol. Cell.* 11: 495-505 (2003), Cho *et al. Nature* 421: 756-760 (2003), Franklin *et al. Cancer Cell* 5:317-328 (2004), or Plowman *et al. Proc. Natl. Acad. Sci.* 90:1746-1750 (1993). See also Fig. 1 herein.

The terms "ErbB1," "HER1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to EGFR as disclosed, for example, in Carpenter *et al. Ann. Rev. Biochem.* 56:881-914 (1987), including naturally occurring mutant forms thereof (*e.g.* a deletion mutant EGFR as in Humphrey *et al. PNAS (USA)* 87:4207-4211 (1990)). *erb*B1 refers to the gene encoding the EGFR protein product.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba *et al.*, *PNAS (USA)* 82:6497-6501 (1985) and Yamamoto *et al. Nature* 319:230-234 (1986) (Genebank accession number X03363). The term "*erb*B2" refers to the gene encoding human ErbB2 and "*neu*" refers to the gene encoding rat p185^{neu}. Preferred HER2 is native sequence human HER2.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al. PNAS (USA)* 86:9193-9197 (1989).

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 WO99/19488, published April 22, 1999.

By "HER ligand" is meant a polypeptide which binds to and/or activates a HER receptor. The HER ligand of particular interest herein is a native sequence human HER ligand such as epidermal growth factor (EGF) (Savage *et al.*, *J. Biol. Chem.* 247:7612-7621 (1972)); transforming growth factor alpha (TGF-α) (Marquardt *et al.*, *Science* 223:1079-1082 (1984)); amphiregulin also known as schwanoma or keratinocyte autocrine growth factor (Shoyab *et al. Science* 243:1074-1076 (1989); Kimura *et al. Nature* 348:257-260 (1990); and Cook *et al. Mol. Cell. Biol.* 11:2547-2557 (1991)); betacellulin (Shing *et al.*, *Science* 259:1604-1607 (1993);

and Sasada *et al. Biochem. Biophys. Res. Commun.* 190:1173 (1993)); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama *et al.*, *Science* 251:936-939 (1991)); epiregulin (Toyoda *et al.*, *J. Biol. Chem.* 270:7495-7500 (1995); and Komurasaki *et al. Oncogene* 15:2841-2848 (1997)); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway *et al.*, *Nature* 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang *et al.*, *Proc. Natl. Acad. Sci.* 94:9562-9567 (1997)); neuregulin-4 (NRG-4) (Harari *et al. Oncogene* 18:2681-89 (1999)) or cripto (CR-1) (Kannan *et al. J. Biol. Chem.* 272(6):3330-3335 (1997)). HER ligands which bind EGFR include EGF, TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. HER ligands which bind HER3 include heregulins. HER ligands capable of binding HER4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins.

"Heregulin" (HRG) when used herein refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Patent No. 5,641,869 or Marchionni *et al.*, *Nature*, 362:312-318 (1993). Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes *et al.*, *Science*, 256:1205-1210 (1992); and U.S. Patent No. 5,641,869); *neu* differentiation factor (NDF) (Peles *et al. Cell* 69: 205-216 (1992)); acetylcholine receptor-inducing activity (ARIA) (Falls *et al. Cell* 72:801-815 (1993)); glial growth factors (GGFs) (Marchionni *et al.*, *Nature*, 362:312-318 (1993)); sensory and motor neuron derived factor (SMDF) (Ho *et al. J. Biol. Chem.* 270:14523-14532 (1995)); γ-heregulin (Schaefer *et al. Oncogene* 15:1385-1394 (1997)). 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.* $HRG\beta1_{177-244}$).

A "HER dimer" herein is a noncovalently associated dimer comprising at least two different HER receptors. Such complexes may form when a cell expressing two or more HER receptors is exposed to an HER ligand and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski *et al.*, *J. Biol. Chem.*, 269(20):14661-14665 (1994), for example. Examples of such HER dimers include EGFR-HER2, HER2-HER3 and HER3-HER4 heterodimers. Moreover, the HER dimer may comprise two or more HER2 receptors combined with a different HER receptor, such as HER3, HER4 or EGFR. Other proteins, such as a cytokine receptor subunit (*e.g.* gp130) may be associated with the dimer.

A "heterodimeric binding site" on HER2, refers to a region in the extracellular domain of HER2 that contacts, or interfaces with, a region in the extracellular domain of EGFR, HER3 or HER4 upon formation of a dimer therewith. The region is found in Domain II of HER2. Franklin *et al. Cancer Cell* 5:317-328 (2004).

"HER activation" or "HER2 activation" refers to activation, or phosphorylation, of any one or more HER receptors, or HER2 receptors. Generally, HER activation results in signal transduction *(e.g.* that caused by an intracellular kinase domain of a HER receptor phosphorylating tyrosine residues in the HER receptor or a substrate polypeptide). HER activation may be mediated by HER ligand binding to a HER dimer comprising the HER receptor of interest. HER ligand binding to a HER dimer may activate a kinase domain of one or more of the HER receptors in the dimer and thereby results in phosphorylation of tyrosine residues in one or more of the HER receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s), such as Akt or MAPK intracellular kinases.

The term "antibody" herein is used in the broadest sense and specifically covers full length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two full length antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

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 and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

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

"Antibody fragments" comprise a portion of a full length antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

A "full length antibody" is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variants thereof. Preferably, the full length antibody has one or more effector functions.

The term "main species antibody" herein refers to the antibody structure in a composition which is the quantitatively predominant antibody molecule in the composition. In one embodiment, the main species antibody is a HER2 antibody, such as an antibody that binds to Domain II of HER2, antibody that inhibits HER dimerization more effectively than Trastuzumab, and/or an antibody which binds to a heterodimeric binding site of HER2. The preferred embodiment herein of a main species HER2 antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 15 and 16 (Pertuzumab).

An "amino acid sequence variant" antibody herein is an antibody with an amino acid sequence which differs from a main species antibody. Ordinarily, amino acid sequence variants will possess at least about 70%

homology with the main species antibody, and preferably, they will be at least about 80%, more preferably at least about 90% homologous with the main species antibody. The amino acid sequence variants possess substitutions, deletions, and/or additions at certain positions within or adjacent to the amino acid sequence of the main species antibody. Examples of amino acid sequence variants herein include acidic variant (*e.g.* deamidated antibody variant), basic variant, the antibody with an amino-terminal leader extension (*e.g.* VHS-) on one or two light chains thereof, antibody with a C-terminal lysine residue on one or two heavy chains thereof, etc, and includes combinations of variations to the amino acid sequences of heavy and/or light chains. The antibody variant of particular interest herein is the antibody comprising an amino-terminal leader extension on one or two light chains thereof, optionally further comprising other amino acid sequence and/or glycosylation differences relative to the main species antibody.

A "therapeutic monoclonal antibody" is an antibody used for therapy of a human subject. Therapeutic monoclonal antibodies disclosed herein include: HER2 antibodies for cancer and various non-malignant diseases or disorders; CD20 or BR3 antibodies for therapy of B cell malignancies, autoimmune diseases, graft rejection, or blocking an immune response to a foreign antigen; IgE antibodies for therapy of an IgE-mediated disorder; DR5 or VEGF antibodies for cancer therapy.

A "glycosylation variant" antibody herein is an antibody with one or more carbohydrate moeities attached thereto which differ from one or more carbohydate moieties attached to a main species antibody. Examples of glycosylation variants herein include antibody with a G1 or G2 oligosaccharide structure, instead a G0 oligosaccharide structure, attached to an Fc region thereof, antibody with one or two carbohydrate moieties attached to one or two light chains thereof, antibody with no carbohydrate attached to one or two heavy chains of the antibody, etc, and combinations of glycosylation alterations.

Where the antibody has an Fc region, an oligosaccharide structure such as that shown in Fig. 16 herein may be attached to one or two heavy chains of the antibody, *e.g.* at residue 299 (298, Eu numbering of residues). For Pertuzumab, G0 was the predominant oligosaccharide structure, with other oligosaccharide structures such as G0-F, G-1, Man5, Man6, G1-1, G1(1-6), G1(1-3) and G2 being found in lesser amounts in the Pertuzumab composition.

Unless indicated otherwise, a "G1 oligosaccharide structure" herein includes G-1, G1-1, G1(1-6) and G1(1-3) structures.

An "amino-terminal leader extension" herein refers to one or more amino acid residues of the amino-terminal leader sequence that are present at the amino-terminus of any one or more heavy or light chains of an antibody. An exemplary amino-terminal leader extension comprises or consists of three amino acid residues, VHS, present on one or both light chains of an antibody variant.

"Homology" 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 homology. 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 "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent

cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor; BCR), etc.

Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different "classes". There are five major classes of full length 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.

"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, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be *assessed in vivo*, *e.g.*, in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, *e.g.* from blood or PBMCs as described herein.

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

"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.*, *J. Immunol. Methods* 202:163 (1996), 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 (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) 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.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). 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.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) 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 *J. Mol. Biol.* 196:901-917 (1987)). "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 V_H-V_L 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" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). HER2 antibody scFv fragments are described in WO93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

"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.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

Humanized HER2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 or Trastuzumab (HERCEPTIN®) as described in Table 3 of U.S. Patent 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319) and humanized 2C4 antibodies as described herein.

For the purposes herein, "Trastuzumab," "HERCEPTIN®," and "huMAb4D5-8" refer to an antibody comprising the light and heavy chain amino acid sequences in SEQ ID NOS. 13 and 14, respectively.

Herein, "Pertuzumab," "rhuMAb 2C4," and "OMNITARG™" refer to an antibody comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectfully. Where Pertuzumab is a full length antibody, it preferably comprises the light chain and heavy chain amino acid sequences in SEQ ID NOS. 15 and 16, respectively.

A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

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

An "agonist antibody" is an antibody which binds to and activates a receptor. Generally, the receptor activation capability of the agonist antibody will be at least qualitatively similar (and may be essentially quantitatively similar) to a native agonist ligand of the receptor. An example of an agonist antibody is one which binds to a receptor in the TNF receptor superfamily, such as DR5, and induces apoptosis of cells expressing the TNF receptor (*e.g.* DR5). Assays for determining induction of apoptosis are described in WO98/51793 and WO99/37684, both of which are expressly incorporated herein by reference.

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. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably 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 sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, 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.

A HER2 antibody which "inhibits HER dimerization more effectively than Trastuzumab" is one which reduces or eliminates HER dimers more effectively (for example at least about 2-fold more effectively) than Trastuzumab. Preferably, such an antibody inhibits HER2 dimerization at least about as effectively as an antibody selected from the group consisting of murine monoclonal antibody 2C4, a Fab fragment of murine monoclonal antibody 2C4, Pertuzumab, and a Fab fragment of Pertuzumab. One can evaluate HER dimerization inhibition by studying HER dimers directly, or by evaluating HER activation, or downstream signaling, which results from HER dimerization, and/or by evaluating the antibody-HER2 binding site, etc. Assays for screening for antibodies with the ability to inhibit HER dimerization more effectively than Trastuzumab are described in Agus *et al. Cancer Cell* 2: 127-137 (2002) and WO01/00245 (Adams *et al.).* By way of example only, one may assay for inhibition of HER dimerization by assessing, for example, inhibition of HER dimer formation (see, *e.g.*, Fig. 1A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002); and WO01/00245); reduction in HER ligand activation of cells which express HER dimers (WO01/00245and Fig. 2A-B of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); blocking of HER ligand binding to cells which express HER dimers (WO01/00245, and Fig. 2E of Agus *et al. Cancer Cell* 2: 127-137 (2002), for example); cell growth inhibition of cancer cells (*e.g.* MCF7, MDA-MD-134, ZR-75-1, MD-MB-175, T-47D cells) which express HER dimers in the presence (or absence) of HER ligand (WO01/00245and Figs. 3A-D of Agus *et al. Cancer Cell* 2: 127-137 (2002), for instance); inhibition of downstream signaling (for instance, inhibition of HRG-dependent AKT phosphorylation or inhibition of HRG- or TGFα- dependent MAPK phosphorylation) (see, WO01/00245, and Fig. 2C-D of Agus et al. *Cancer Cell* 2: 127-137 (2002), for example). One may also assess whether the antibody inhibits HER dimerization by studying the antibody-HER2 binding site, for instance, by evaluating a structure or model, such as a crystal structure, of the antibody bound to HER2 (See, for example, Franklin *et al. Cancer Cell* 5:317-328 (2004)).

The HER2 antibody may "inhibit HRG-dependent AKT phosphorylation" and/or inhibit "HRG- or TGFα-dependent MAPK phosphorylation" more effectively (for instance at least 2-fold more effectively) than Trastuzumab (see Agus *et al. Cancer Cell* 2: 127-137 (2002) and WO01/00245, by way of example).

The HER2 antibody may be one which does "not inhibit HER2 ectodomain cleavage" (Molina *et al. Cancer Res.* 61:4744-4749(2001).

A HER2 antibody that "binds to a heterodimeric binding site" of HER2, binds to residues in domain II (and optionally also binds to residues in other of the domains of the HER2 extracellular domain, such as domains I and III), and can sterically hinder, at least to some extent, formation of a HER2-EGFR, HER2-HER3, or HER2-HER4 heterodimer. Franklin *et al. Cancer Cell* 5:317-328 (2004) characterize the HER2-Pertuzumab crystal structure, deposited with the RCSB Protein Data Bank (ID Code 1S78), illustrating an exemplary antibody that binds to the heterodimeric binding site of HER2.

An antibody that "binds to domain II" of HER2 binds to residues in domain II and optionally residues in other domain(s) of HER2, such as domains I and III. Preferably the antibody that binds to domain II binds to the junction between domains I, II and III of HER2.

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

agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

Examples of "growth inhibitory" antibodies are those which bind to HER2 and inhibit the growth of cancer cells overexpressing HER2. Preferred growth inhibitory HER2 antibodies inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, and preferably 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 (see U.S. Patent No. 5,677,171 issued October 14, 1997). The SK-BR-3 cell growth inhibition assay is described in more detail in that patent and hereinbelow. The preferred growth inhibitory antibody is a humanized variant of murine monoclonal antibody 4D5, *e.g.*, Trastuzumab.

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 expresses the antigen to which the antibody binds. Preferably the cell is a tumor cell. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay using cells that express an antigen to which the antibody binds. Examples of antibodies that induce apoptosis are HER2 antibodies 7C2 and 7F3, and certain DR5 antibodies.

The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2. Epitope 2C4 comprises residues from domain II in the extracellular domain of HER2. 2C4 and Pertuzumab bind to the extracellular domain of HER2 at the junction of domains I, II and III. Franklin *et al. Cancer Cell* 5:317-328 (2004).

The "epitope 4D5" is the region in the extracellular domain of HER2 to which the antibody 4D5 (ATCC CRL 10463) and Trastuzumab bind. This epitope is close to the transmembrane domain of HER2, and within Domain IV of HER2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 4D5 epitope of HER2 (*e.g.* any one or more residues in the region from about residue 529 to about residue 625, inclusive, of HER2).

The "epitope 7C2/7F3" is the region at the amino terminus, within Domain I, of the extracellular domain of HER2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below) bind. To screen for antibodies which bind to the 7C2/7F3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2/7F3 epitope on HER2 (*e.g.* any one or more of residues in the region from about residue 22 to about residue 53 of HER2).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disease as well as those in which the disease is to be prevented. Hence, the patient to be treated herein may have been diagnosed as having the disease or may be predisposed or susceptible to the disease.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma (including medulloblastoma and retinoblastoma), sarcoma (including liposarcoma and synovial cell sarcoma), neuroendocrine tumors (including carcinoid tumors, gastrinoma,and islet cell cancer), mesothelioma, schwannoma (including acoustic neuroma), meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, tumors of the biliary tract, as well as head and neck cancer.

The term "effective amount" refers to an amount of a drug effective to a disease in the patient. Where the disease is cancer, the effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. The effective amount may extend progression free survival, result in an objective response (including a partial response, PR, or complete response, CR), increase overall survival time, and/or improve one or more symptoms of cancer.

A "HER2-expressing cancer" is one comprising cells which have HER2 protein present at their cell surface.

A cancer which "overexpresses" a HER receptor is one which has significantly higher levels of a HER receptor, such as HER2, 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. HER receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the HER protein present on the surface of a cell (*e.g.* via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of HER-encoding nucleic acid in the cell, *e.g.* via fluorescent *in situ*

hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study HER receptor overexpression by measuring shed antigen (*e.g.*, HER extracellular domain) in a biological fluid such as serum (see, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias *et al. J. Immunol. Methods* 132: 73-80 (1990)). Aside from the above assays, various *in vivo* assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.* a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.* by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

Conversely, a cancer which "does not overexpress HER2 receptor" is one which does not express higher than normal levels of HER2 receptor compared to a noncancerous cell of the same tissue type.

A cancer which "overexpresses" a HER ligand is one which produces significantly higher levels of that ligand compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. Overexpression of the HER 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.

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.* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} 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 fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; 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 (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores),

aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), peglylated liposomal doxorubicin (CAELYX®), 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, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, 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; 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; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin, and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovovin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (*e.g.*, LURTOTECAN®); rmRH (*e.g.*, ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (Pfizer); perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin,

vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrazole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors including vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, imidazole; lutenizing hormone-releasing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and tripterelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretionic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; testolactone; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

As used herein, the term "EGFR-targeted drug" refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO98/50433, Abgenix). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659,439A2, Merck Patent GmbH). Examples of small molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), CP-358774 or Erlotinib HCL (TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen).

A "tyrosine kinase inhibitor"is a molecule which inhibits to some extent tyrosine kinase activity of a tyrosine kinase such as a HER receptor. Examples of such inhibitors include the EGFR-targeted drugs noted in the preceding paragraph as well as small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda, dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 & EGFR-overexpressing cells, GW572016 (available from Glaxo) an oral HER2 and EGFR tyrosine kinase inhibitor, and PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibits Raf-1 signaling; non-HER targeted TK inhibitors such as Imatinib mesylate (Gleevac™) available from Glaxo; MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines;

pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules *(e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER 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); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: US Patent No. 5,804,396; WO99/09016 (American Cyanimid); WO98/43960 (American Cyanamid); WO97/38983 (Warner Lambert); WO99/06378 (Warner Lambert); WO99/06396 (Warner Lambert); WO96/30347 (Pfizer, Inc); WO96/33978 (Zeneca); WO96/3397 (Zeneca); and WO96/33980 (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. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF), such as Bevacizumab (AVASTIN®).

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 antibody which is formulated is preferably essentially pure and desirably essentially homogeneous (*i.e.* free from contaminating proteins etc). "Essentially pure" antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antibody that binds thereto. Exemplary B-cell surface markers include the

CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers (for descriptions, see The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Other B-cell surface markers include RP105, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest herein is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface marker herein is CD20 or BR3.

The "CD20" antigen, or "CD20," is an about 35-kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is present on both normal B cells as well as malignant B cells, but is not expressed on stem cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al.* *Proc. Natl. Acad. Sci.* (USA) 82:1766 (1985), for example.

Purely for the purposes herein, "humanized 2H7" refers to a humanized variant of the 2H7 antibody whose CDR sequences are disclosed in US Patent No. 5,500,362 (Figs. 5 and 6), expressly incorporated herein by reference. Examples of humanized 2H7 antibodies herein include the variants described in WO2004/056312, also expressly incorporated herein by reference, as well as other variants, including, but not limited to: 2H7v16, 2H7v31, 2H7v73, 2H7v75, 2H7v96, 2H7v114, 2H7v115, 2H7v116, 2H7v138, 2H7v477, 2H7v375, etc.

In one embodiment, the humanized 2H7 antibody comprises one, two, three, four, five or six of the following CDR sequences:

CDR L1 sequence RASSSVSYXH wherein X is M or L (SEQ ID No. 67), for example SEQ ID No. 57 (Fig. 18A),

CDR L2 sequence of SEQ ID No. 58 (Fig. 18A),

CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID No. 68), for example SEQ ID No. 59 (Fig. 18A),

CDR H1 sequence of SEQ ID No. 60 (Fig. 18B),

CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID No. 69), for example SEQ ID No. 61 (Fig. 18B), and

CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W or D, and the X at position 7 is S or R (SEQ ID No. 70), for example SEQ ID No. 62 (Fig. 18B).

The CDR sequences above are generally present within human variable light and variable heavy framework sequences, such as substantially the human consensus FR residues of human light chain kappa subgroup I (V_L6I), and substantially the human consensus FR residues of human heavy chain subgroup III (V_HIII). See also WO 2004/056312 (Lowman *et al.*).

The variable heavy region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3, including native sequence and variant constant regions.

In a preferred embodiment, such antibody comprises the variable heavy domain sequence of SEQ ID No. 29 (v16, as shown in Fig. 18B), optionally also comprising the variable light domain sequence of SEQ ID No. 26 (v16, as shown in Fig. 18A), which optionally comprises one or more amino acid substitution(s) at positions 56, 100, and/or 100a, *e.g.* D56A, N100A or N100Y, and/or S100aR in the variable heavy domain and

one or more amino acid substitution(s) at positions 32 and/or 92, *e.g.* M32L and/or S92A, in the variable light domain. Preferably, the antibody is an intact antibody comprising the light chain amino acid sequences of SEQ ID Nos. 63 or 64, and heavy chain amino acid sequences of SEQ ID No. 65, 66, 71 or 72.

A preferred humanized 2H7 antibody is ocrelizumab (Genentech).

The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC activity, such as one wherein the amino acid substitutions are at positions 298, 333, and 334, preferably S298A, E333A, and K334A, using Eu numbering of heavy chain residues. See also US Patent No. 6,737,056B1, Presta.

Any of these antibodies may comprise at least one substitution in the Fc region that improves FcRn binding or serum half-life, for example a substitution at heavy chain position 434, such as N434W. See also US Patent No. 6,737,056B1, Presta.

Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that increases CDC activity, for example, comprising at least a substitution at position 326, preferably K326A or K326W. See also US Patent No. 6,528,624B1 (Idusogie *et al.*).

Some preferred humanized 2H7 variants are those comprising the variable light domain of SEQ ID No. 26 and the variable heavy domain of SEQ ID No. 29, including those with or without substitutions in an Fc region (if present), and those comprising a variable heavy domain with alteration N100A; or D56A and N100A; or D56A, N100Y, and S100aR; in SEQ ID No. 29 and a variable light domain with alteration M32L; or S92A; or M32L and S92A; in SEQ ID No. 26.

M34 in the variable heavy chain of 2H7v16 has been identified as a potential source of antibody stability and is another potential candidate for substitution.

In a summary of some various preferred embodiments of the invention, the variable region of variants based on 2H7v16 comprise the amino acid sequences of v16 except at the positions of amino acid substitutions that are indicated in the Table below. Unless otherwise indicated, the 2H7 variants will have the same light chain as that of v16.

Exemplary Humanized 2H7 Antibody Variants

2H7 Version	Heavy chain (V_H) changes	Light chain (V_L) changes	Fc changes
16 for reference			-
31	-	-	S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N100A	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K334A, K322A
138	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A
477	D56A, N100A	M32L, S92A	S298A, E333A, K334A, K326A, N434W
375	-	-	K334L
588	-	-	S298A, E333A, K334A, K326A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, E333A, K334A, K326A

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One preferred humanized 2H7 comprises 2H7v16 variable light domain sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID No. 26);

and 2H7v16 variable heavy domain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID No. 29).

Where the humanized 2H7v16 antibody is an intact antibody, it may comprise the light chain amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID No. 63):

and the heavy chain amino acid sequence of SEQ ID No. 65 or:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 71).

Another preferred humanized 2H7 antibody comprises 2H7v511 variable light domain sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID No. 73)

and 2H7v511 variable heavy domain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS (SEQ ID No. 74).

Where the humanized 2H7v511 antibody is an intact antibody, it may comprise the light chain amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC (SEQ ID No. 64)

and the heavy chain amino acid sequence of SEQ ID No. 66 or:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAA
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID No. 72).

A "B-cell malignancy" herein includes non-Hodgkin's lymphoma (NHL), including low grade/follicular NHL, small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia; leukemia, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia and chronic myeloblastic leukemia; and other hematologic malignancies. Such malignancies may be treated with antibodies directed against B-cell surface markers, such as CD20.

The term "non-Hodgkin's lymphoma" or "NHL", as used herein, refers to a cancer of the lymphatic system other than Hodgkin's lymphomas. Hodgkin's lymphomas can generally be distinguished from non-Hodgkin's lymphomas by the presence of Reed-Sternberg cells in Hodgkin's lymphomas and the absence of said cells in non-Hodgkin's lymphomas. Examples of non-Hodgkin's lymphomas encompassed by the term as used herein include any that would be identified as such by one skilled in the art (*e.g.*, an oncologist or pathologist) in accordance with classification schemes known in the art, such as the Revised European-American Lymphoma (REAL) scheme as described in Color Atlas of Clinical Hematology, Third Edition; A. Victor Hoffbrand and John E. Pettit (eds.) (Harcourt Publishers Limited 2000) (see, in particular Fig. 11.57, 11.58 and/or 11.59). More specific examples include, but are not limited to, relapsed or refractory NHL, front line low grade NHL, Stage III/IV NHL, chemotherapy resistant NHL, precursor B lymphoblastic leukemia and/or lymphoma, small lymphocytic lymphoma, B cell chronic lymphocytic leukemia and/or prolymphocytic leukemia and/or small lymphocytic lymphoma, B-cell prolymphocytic lymphoma, immunocytoma and/or lymphoplasmacytic lymphoma, marginal zone B cell lymphoma, splenic marginal zone lymphoma, extranodal marginal zone - MALT lymphoma, nodal marginal zone lymphoma, hairy cell leukemia, plasmacytoma and/or plasma cell myeloma, low grade/follicular lymphoma, intermediate grade/follicular NHL, mantle cell lymphoma, follicle center lymphoma (follicular), intermediate grade diffuse NHL, diffuse large B-cell lymphoma, aggressive NHL (including aggressive front-line NHL and aggressive relapsed NHL), NHL relapsing after or refractory to autologous stem cell transplantation, primary mediastinal large B-cell lymphoma, primary effusion lymphoma, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, Burkitt's lymphoma, precursor (peripheral) T-cell lymphoblastic leukemia and/or lymphoma, adult T-cell lymphoma and/or leukemia, T cell chronic lymphocytic leukemia and/or prolymphacytic leukemia, large granular lymphocytic leukemia, mycosis fungoides and/or Sezary syndrome, extranodal natural killer/T-cell (nasal type) lymphoma, enteropathy type T-cell lymphoma, hepatosplenic T-cell lymphoma, subcutaneous panniculitis like T-cell lymphoma, skin (cutaneous) lymphomas, anaplastic large cell lymphoma, angiocentric lymphoma, intestinal T cell lymphoma, peripheral T-cell (not otherwise specified) lymphoma and angioimmunoblastic T-cell lymphoma.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting condition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis, juvenile-onset rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis, chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, scleroderma (including systemic scleroderma), sclerosis such as progressive systemic sclerosis, inflammatory bowel disease (IBD) (for example, Crohn's

disease, ulcerative colitis, autoimmune inflammatory bowel disease), pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic and atopic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis or autoimmune uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN (membranous nephropathy), idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, allergic reaction, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, subacute cutaneous lupus erythematosus, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset (Type I) diabetes mellitus, including pediatric insulin-dependent diabetes mellitus (IDDM), adult onset diabetes mellitus (Type II diabetes), multiple sclerosis (MS) such as spino-optical MS, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including lymphomatoid granulomatosis, Wegener's granulomatosis, agranulocytosis, vasculitis (including large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), CNS vasculitis, systemic necrotizing vasculitis, and ANCA-associated vasculitis , such as Churg-Strauss vasculitis or syndrome (CSS)), temporal arteritis, aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, hemolytic anemia or immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet's or Behcet's disease, Castleman's syndrome, Goodpasture's syndrome, Reynaud's syndrome, Sjogren's syndrome, Stevens-Johnson syndrome, pemphigoid such as pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's disease, immune complex nephritis, chronic neuropathy such as IgM polyneuropathies or IgM-mediated neuropathy, thrombocytopenia (as developed by myocardial infarction patients, for example), including thrombotic thrombocytopenic purpura (TTP) and autoimmune or immune-mediated thrombocytopenia such as idiopathic thrombocytopenic purpura (ITP) including chronic or acute ITP, autoimmune disease of the testis and ovary including autoimune orchitis and oophoritis, primary hypothyroidism, hypoparathyroidism, autoimmune endocrine diseases including thyroiditis such as autoimmune thyroiditis, chronic thyroiditis (Hashimoto's thyroiditis), or subacute thyroiditis, autoimmune thyroid disease, idiopathic hypothyroidism, Addison's disease, Grave's disease, polyglandular syndromes such as autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), paraneoplastic syndromes, including neurologic paraneoplastic syndromes such as Lambert-Eaton myasthenic syndrome or Eaton-Lambert syndrome, stiff-man or stiff-person syndrome, encephalomyelitis such as allergic encephalomyelitis, myasthenia gravis, cerebellar degeneration, limbic and/or brainstem encephalitis, neuromyotonia, opsoclonus or opsoclonus myoclonus syndrome (OMS), and sensory neuropathy, Sheehan's syndrome, autoimmune hepatitis, chronic hepatitis, lupoid hepatitis, chronic active hepatitis or autoimmune chronic active hepatitis, lymphoid interstitial pneumonitis, bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré syndrome, Berger's disease (IgA nephropathy), primary biliary cirrhosis,

celiac sprue (gluten enteropathy), refractory sprue, dermatitis herpetiformis, cryoglobulinemia, amylotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), or autoimmune hearing loss, opsoclonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, a non-cancerous lymphocytosis, a primary lymphocytosis, which includes monoclonal B cell lymphocytosis (e.g., benign monoclonal gammopathy and monoclonal gammopathy of undetermined significance, MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS, autism, inflammatory myopathy, focal segmental glomerulosclerosis (FSGS), endocrine ophthalmopathy, uveoretinitis, autoimmune hepatological disorder, fibromyalgia, multiple endocrine failure, Schmidt's syndrome, adrenalitis, gastric atrophy, presenile dementia, demyelinating diseases, Dressler's syndrome, alopecia areata, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, mixed connective tissue disease, Chagas' disease, rheumatic fever, recurrent abortion, farmer's lung, erythema multiforme, post-cardiotomy syndrome, Cushing's syndrome, bird-fancier's lung, Alport's syndrome, alveolitis such as allergic alveolitis and fibrosing alveolitis, interstitial lung disease, transfusion reaction, leprosy, malaria, leishmaniasis, kypanosomiasis, schistosomiasis, ascariasis, aspergillosis, Sampter's syndrome, Caplan's syndrome, dengue, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, flariasis, cyclitis such as chronic cyclitis, heterochronic cyclitis, or Fuch's cyclitis, Henoch-Schonlein purpura, human immunodeficiency virus (HIV) infection, echovirus infection, cardiomyopathy, Alzheimer's disease, parvovirus infection, rubella virus infection, post-vaccination syndromes, congenital rubella infection, Epstein-Barr virus infection, mumps, Evan's syndrome, autoimmune gonadal failure, Sydenham's chorea, post-streptococcal nephritis, thromboangitis ubiterans, thyrotoxicosis, tabes dorsalis, and giant cell polymyalgia.

The "tumor necrosis factor receptor superfamily" or "TNF receptor superfamily" herein refers to receptor polypeptides bound by cytokines in the TNF family. Generally, these receptors are Type I transmembrane receptors with one or more cysteine rich repeat sequences in their extracellular domain. The TNF receptor superfamily may be further subdivided into (1) death receptors; (2) decoy receptors; and (3) signaling receptors that lack death domains. The "death receptors" contain in their cytoplasmic or intracellular region a "death domain", *i.e.*, a region or sequence which acts to transduce signals in the cell which can result in apoptosis or in induction of certain genes. The "decoy receptors" lack a functional death domain and are incapable of transducing signals which result in apoptosis. Examples of cytokines in the TNF gene family include Tumor Necrosis Factor- alpha (TNF-alpha), Tumor Necrosis Factor-beta (TNF-beta or lymphotoxin), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as TRAIL), Apo-3 ligand (also referred to as TWEAK), osteoprotegerin (OPG), APRIL, RANK ligand (also referred to as TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK). Examples of receptors in the TNF receptor superfamily include: type 1 Tumor Necrosis Factor Receptor (TNFR1), type 2 Tumor Necrosis Factor Receptor (TNFR2), p75 Nerve Growth Factor receptor (NGFR), the B cell surface antigen CD40, the T cell antigen OX-40, Apo-1 receptor (also called Fas or CD95), Apo-3 receptor (also called DR3, swl-1, TRAMP and LARD), the receptor called "Transmembrane

Activator and CAML-Interactor" or "TACI", BCMA protein, DR4, DR5 (alternatively referred to as Apo-2; TRAIL-R2, TR6, Tango-63, hAPO8, TRICK2 or KILLER), DR6, DcR1 (also referred to as TRID, LIT or TRAIL-R3), DcR2 (also called TRAIL-R4 or TRUNDD), OPG, DcR3 (also called TR6 or M68), CAR1, HVEM (also called ATAR or TR2), GITR, ZTNFR-5, NTR-1, TNFL1, CD30, Lymphotoxin beta receptor (LTBr), 4-1BB receptor and TR9 (EP988.371A1).

The terms "Apo-2 ligand", "Apo-2L", "Apo2L", Apo-2 ligand/TRAIL" and "TRAIL" are used herein interchangeably to refer to a polypeptide sequence which includes amino acid residues 114-281, inclusive, 95-281, inclusive, residues 92-281, inclusive, residues 91-281, inclusive, residues 41-281, inclusive, residues 39-281, inclusive, residues 15-281, inclusive, or residues 1-281, inclusive, of the amino acid sequence shown in Fig. 24 (SEQ ID No. 46), as well as biologically active fragments, deletional, insertional, and/or substitutional variants of the above sequences. In one embodiment, the polypeptide sequence comprises residues 114-281 of Fig. 24 (SEQ ID No. 46). Optionally, the polypeptide sequence comprises residues 92-281 or residues 91-281 of Fig. 24 (SEQ ID No. 46). The Apo-2L polypeptides may be encoded by the native nucleotide sequence shown in Fig. 24 (SEQ ID No. 45). Optionally, the codon which encodes residue Pro119 (Fig. 24; SEQ ID No. 45) may be "CCT" or "CCG". Optionally, the fragments or variants are biologically active and have at least about 80% amino acid sequence identity, or at least about 90% sequence identity, or at least 95%, 96%, 97%, 98%, or 99% sequence identity with any one of the above sequences. The definition encompasses substitutional variants of Apo-2 ligand in which at least one of its native amino acids are substituted by another amino acid such as an alanine residue. The definition also encompasses a native sequence Apo-2 ligand isolated from an Apo-2 ligand source or prepared by recombinant and/or synthetic methods. The Apo-2 ligand of the invention includes the polypeptides referred to as Apo-2 ligand or TRAIL disclosed in WO97/01633 published January 16, 1997, WO97/25428 published July 17, 1997, WO99/36535 published July 22, 1999, WO 01/00832 published January 4, 2001, WO02/09755 published February 7, 2002, WO 00/75191 published December 14, 2000, and U.S. Patent No. 6,030,945 issued February 29, 2000. The terms are used to refer generally to forms of the Apo-2 ligand which include monomer, dimer, trimer, hexamer or hight oligomer forms of the polypeptide. All numbering of amino acid residues referred to in the Apo-2L sequence use the numbering according to Fig. 24 (SEQ ID No. 46), unless specifically stated otherwise.

"Apo-2 ligand receptor" includes the receptors referred to in the art as "DR4" and "DR5." *Pan et al.* have described the TNF receptor family member referred to as "DR4" (Pan *et al.*, *Science*, 276:111-113 (1997); see also WO98/32856 published July 30, 1998; WO 99/37684 published July 29, 1999; WO 00/73349 published December 7, 2000; US 6,433,147 issued August 13, 2002; US 6,461,823 issued October 8, 2002, and US 6,342,383 issued January 29, 2002). Sheridan *et al.*, *Science*, 277:818-821 (1997) and Pan *et al.*, *Science*, 277:815-818 (1997) described another receptor for Apo2L/TRAIL (see also, WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998). This receptor is referred to as DR5 (the receptor has also been alternatively referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER; Screaton *et al.*, *Curr. Biol.*, 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924

published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003). As described above, other receptors for Apo-2L include DcR1, DcR2, and OPG. The term "Apo-2L receptor" when used herein encompasses native sequence receptor and receptor variants. These terms encompass Apo-2L receptor expressed in a variety of mammals, including humans. Apo-2L receptor may be endogenously expressed as occurs naturally in a variety of human tissue lineages, or may be expressed by recombinant or synthetic methods. A "native sequence Apo-2L receptor" comprises a polypeptide having the same amino acid sequence as an Apo-2L receptor derived from nature. Thus, a native sequence Apo-2L receptor can have the amino acid sequence of naturally-occurring Apo-2L receptor from any mammal, including humans. Such native sequence Apo-2L receptor can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence Apo-2L receptor" specifically encompasses naturally-occurring truncated or secreted forms of the receptor (*e.g.*, a soluble form containing, for instance, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants. Receptor variants may include fragments or deletion mutants of the native sequence Apo-2L receptor. Figs. 25A-C show the 411 amino acid sequence of human DR5 receptor, along with its nucleotide sequence (SEQ ID Nos. 47 and 48) as published in WO 98/51793 on November 19, 1998. A transcriptional splice variant of human DR5 receptor is known in the art. This splice variant encodes the 440 amino acid sequence of human DR5 receptor as shown in Figs. 26A-C, along with its nucleotide sequence (SEQ ID Nos. 49 and 50), and as published in WO 98/35986 on August 20, 1998.

"Death receptor antibody" is used herein to refer generally to antibody or antibodies directed to a receptor in the tumor necrosis factor receptor superfamily and containing a death domain capable of signalling apoptosis, and such antibodies include DR5 antibody and DR4 antibody.

"DR5 receptor antibody", "DR5 antibody", or "anti-DR5 antibody" is used in a broad sense to refer to antibodies that bind to at least one form of a DR5 receptor or extracellular domain thereof. Optionally the DR5 antibody is fused or linked to a heterologous sequence or molecule. Preferably the heterologous sequence allows or assists the antibody to form higher order or oligomeric complexes. Optionally, the DR5 antibody binds to DR5 receptor but does not bind or cross-react with any additional Apo-2L receptor (*e.g.* DR4, DcR1, or DcR2). Optionally the antibody is an agonist of DR5 signalling activity.

Optionally, the DR5 antibody of the invention binds to a DR5 receptor at a concentration range of about 0.1 nM to about 20 mM as measured in a BIAcore binding assay. Optionally, the DR5 antibodies of the invention exhibit an IC50 value of about 0.6 nM to about 18 mM as measured in a BIAcore binding assay.

Purely for the purposes herein, the term "Apomab" refers to an agonist antibody which binds to DR5 and comprises the variable heavy and variable light amino acid sequences of SEQ ID Nos. 55 and 56. Preferably Apomab comprises the heavy and light chains of SEQ ID Nos. 51 and 52, respectively.

II. Production of Antibodies

Techniques for producing antibodies which can be formulated according to the present invention follow.

(i) Antigen selection and preparation

Preferably, the antigen to which the antibody binds is a biologically important glycoprotein and administration of the antibody to a mammal suffering from a disease or disorder can result in a therapeutic benefit in that mammal. However, antibodies directed against nonpolypeptide antigens (such as tumor-associated glycolipid antigens: see US Patent 5,091,178) are also contemplated.

Where the antigen is a polypeptide, it may be a transmembrane molecule (*e.g.* receptor) or ligand such as a growth factor. Exemplary antigens include molecules such as renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor (TF), and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-b; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-b1, TGF-b2, TGF-b3, TGF-b4, or TGF-b5; a tumor necrosis factor (TNF) such as TNF-alpha or TNF-beta; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19, CD20, CD22 and CD40; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9 and IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4 and VCAM; a tumor associated antigen such as HER2, HER3 or HER4 receptor; and fragments of any of the above-listed polypeptides.

Exemplary molecular targets for antibodies encompassed by the present invention include CD proteins such as CD3, CD4, CD8, CD19, CD20, CD22, CD34 and CD40; members of the ErbB receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; B cell surface antigens, such as CD20 or BR3; a member of the tumor necrosis receptor superfamily, including DR5; prostate stem cell antigen (PSCA); cell adhesion molecules such as LFA-1, Mac1, p150.95, VLA-4, ICAM-1, VCAM, alpha4/beta7 integrin, and alphav/beta3

integrin including either alpha or beta subunits thereof (*e.g.* anti-CD11a, anti-CD18 or anti-CD11b antibodies); growth factors such as VEGF as well as receptors therefor; tissue factor (TF); a tumor necrosis factor (TNF) such as TNF-alpha or TNF-beta, alpha interferon (alpha-IFN); an interleukin, such as IL-8; IgE; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; mpl receptor; CTLA-4; protein C etc.

Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (*e.g.* the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (*e.g.* cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule. Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

For production of HER2 antibodies, the HER2 antigen to be used for production thereof may be, *e.g.*, a soluble form of the extracellular domain of HER2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing HER2 at their cell surface (*e.g.* NIH-3T3 cells transformed to overexpress HER2; or a carcinoma cell line such as SK-BR-3 cells, see Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991)) can be used to generate antibodies.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

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

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11

mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); 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. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

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 preferred 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. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

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.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) 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.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). 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 (U.S. Patent No. 4,816,567;

and Morrison, *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), 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.

(iii) Humanized antibodies

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

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

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

WO01/00245 describes production of exemplary humanized HER2 antibodies which bind HER2 and block ligand activation of a HER receptor. The humanized antibody of particular interest herein blocks EGF, TGF-α and/or HRG mediated activation of MAPK essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof) and/or binds HER2 essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof). The humanized antibody herein 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.

An exemplary humanized antibody of interest herein comprises variable heavy domain complementarity determining residues GFTFTDYTMX, where X is preferably D or S (SEQ ID No. 7); DVNPNSGGSIYNQRFKG (SEQ ID No. 8); and/or NLGPSFYFDY (SEQ ID No. 9), optionally comprising amino acid modifications of those CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable heavy CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable heavy domain amino acid sequence in SEQ ID No. 4.

The humanized antibody may comprise variable light domain complementarity determining residues KASQDVSIGVA (SEQ ID No. 10); SASYXXX, where the X as position 5 is preferably R or L, wherein the X at position 6 is preferably Y or E, and the X as position 7 is preferably T or S (SEQ ID No. 11); and/or QQYYIYPYT (SEQ ID No. 12), *e.g.* in addition to those variable heavy domain CDR residues in the preceding paragraph. Such humanized antibodies optionally comprise amino acid modifications of the above CDR residues, *e.g.* where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable light CDR sequences. Such antibody variants may be prepared by affinity maturation, *e.g.*, as described below. The most preferred humanized antibody comprises the variable light domain amino acid sequence in SEQ ID No. 3.

The present application also contemplates affinity matured antibodies which bind HER2 and block ligand activation of a HER receptor. The parent antibody may be a human antibody or a humanized antibody, *e.g.*, one comprising the variable light and/or heavy sequences of SEQ ID Nos. 3 and 4, respectively (*i.e.* variant 574). The affinity matured antibody preferably binds to HER2 receptor with an affinity superior to that of murine 2C4 or variant 574 (*e.g.* from about two or about four fold, to about 100 fold or about 1000 fold improved affinity, *e.g.* as assessed using a HER2-extracellular domain (ECD) ELISA). Exemplary variable heavy CDR residues for substitution include H28, H30, H34, H35, H64, H96, H99, or combinations of two or more (*e.g.* two, three, four, five, six, or seven of these residues). Examples of variable light CDR residues for alteration include L28, L50, L53, L56, L91, L92, L93, L94, L96, L97 or combinations of two or more (*e.g.* two to three, four, five or up to about ten of these residues).

Various forms of the humanized antibody or affinity matured antibody are contemplated. For example, the humanized antibody or affinity matured antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody or affinity matured antibody may be an full length antibody, such as an full length IgG1 antibody.

(iv) Human antibodies

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. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993); and U.S. Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. 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 following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

Human HER2 antibodies are described in U.S. Patent No. 5,772,997 issued June 30, 1998 and WO 97/00271 published January 3, 1997.

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of full length antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)).

However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. 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; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the HER2 protein. Other such antibodies may combine a HER2 binding site with binding site(s) for EGFR, HER3 and/or HER4. Alternatively, a HER2 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 HER2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express HER2. These antibodies possess a HER2-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies).

WO 96/16673 describes a bispecific HER2/FcγRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific HER2/FcγRI antibody IDM1 (Osidem). A bispecific HER2/Fcα antibody is shown in WO98/02463. U.S. Patent No. 5,821,337 teaches a bispecific HER2/CD3 antibody. MDX-210 is a bispecific HER2-FcγRIII Ab.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

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

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

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

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein full length antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the

production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

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

(vii) Other amino acid sequence modifications

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the Antibody are prepared by introducing appropriate nucleotide changes into the Antibody nucleic acid, 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 called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed Antibody variants are screened for the desired activity.

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

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

Table 1

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

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to

similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

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

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

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

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

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

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

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

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

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

(6) aromatic: Trp, Tyr, Phe.

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

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).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and its antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

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

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 A1, Presta, L. See also US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO03/011878, Jean-Mairet *et al.* and US Patent No. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO97/30087, Patel *et al.* See, also, WO98/58964 (Raju, S.) and WO99/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. Antibody compositions comprising main species antibody with such carbohydrate structures attached to the Fc region are contemplated herein.

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.

(viii) Screening for antibodies with the desired properties

Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

To identify an antibody which blocks ligand activation of a HER receptor, the ability of the antibody to block HER ligand binding to cells expressing the HER receptor (*e.g.* in conjugation with another HER receptor with which the HER receptor of interest forms a HER hetero-oligomer) may be determined. For example, cells naturally expressing, or transfected to express, HER receptors of the HER hetero-oligomer may be incubated with the antibody and then exposed to labeled HER ligand. The ability of the HER2 antibody to block ligand binding to the HER receptor in the HER hetero-oligomer may then be evaluated.

For example, inhibition of HRG binding to MCF7 breast tumor cell lines by HER2 antibodies may be performed using monolayer MCF7 cultures on ice in a 24-well-plate format essentially as described in WO01/00245. HER2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRG$\beta 1_{177-224}$ (25 pm) 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 antibody of interest. In one embodiment, the antibody which blocks ligand activation of an HER receptor will have an IC_{50} for inhibiting HRG binding to MCF7 cells in this assay of about 50nM or less, more preferably 10nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG binding to MCF7 cells in this assay may, for example, be about 100nM or less, more preferably 50nM or less.

Alternatively, or additionally, the ability of the HER2 antibody to block HER ligand-stimulated tyrosine phosphorylation of a HER receptor present in a HER hetero-oligomer may be assessed. For example, cells endogenously expressing the HER receptors or transfected to expressed them may be incubated with the antibody and then assayed for HER ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S. Patent No. 5,766,863 is also available for determining HER receptor activation and blocking of that activity by an antibody.

In one embodiment, one may screen for an antibody which inhibits HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described in WO01/00245. For example, the MCF7 cells may be plated in 24-well plates and monoclonal antibodies to HER2 may be added to each well and incubated for 30 minutes at room temperature; then rHRGβ1$_{177-244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for 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. The antibody selected will preferably significantly inhibit HRG stimulation of p180 tyrosine phosphorylation to about 0-35% of 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 antibody of interest may be calculated. In one embodiment, the antibody which blocks ligand activation of a HER receptor will have an IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay of about 50nM or less, more preferably 10nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay may, for example, be about 100nM or less, more preferably 50nM or less.

One may also assess the growth inhibitory effects of the antibody on MDA-MB-175 cells, *e.g.* essentially as described in Schaefer *et al. Oncogene* 15:1385-1394 (1997). According to this assay, MDA-MB-175 cells may treated with a HER2 monoclonal antibody (10µg/mL) for 4 days and stained with crystal violet. Incubation with a HER2 antibody may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4. In a further embodiment, exogenous HRG will not significantly reverse this inhibition. Preferably, the antibody will be able to inhibit cell proliferation of MDA-MB-175 cells to a greater extent than monoclonal antibody 4D5 (and optionally to a greater extent than monoclonal antibody 7F3), both in the presence and absence of exogenous HRG.

In one embodiment, the HER2 antibody of interest may block heregulin dependent association of HER2 with HER3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment such as that described in WO01/00245 substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

To identify growth inhibitory HER2 antibodies, one may screen for antibodies which inhibit the growth of cancer cells which overexpress HER2. In one embodiment, the growth inhibitory antibody of choice is able to inhibit growth of SK-BR-3 cells in cell culture by about 20-100% and preferably by about 50-100% at an antibody concentration of about 0.5 to 30 μg/ml. To identify such antibodies, the SK-BR-3 assay described in U.S. Patent No. 5,677,171 can be performed. According to this assay, SK-BR-3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The SK-BR-3 cells are plated at 20,000 cells in a 35mm cell culture dish (2mls/35mm dish). 0.5 to 30 μg/ml of the HER2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SK-BR-3 cells by about 20-100% or about 50-100% may be selected as growth inhibitory antibodies. See US Pat No. 5,677,171 for assays for screening for growth inhibitory antibodies, such as 4D5 and 3E8.

In order to select for HER2 antibodies which induce apoptosis, an annexin binding assay using BT474 cells is available. The BT474 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 10μg/ml of the monoclonal antibody. 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-FTIC) (1μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing antibodies. In addition to the annexin binding assay, a DNA staining assay using BT474 cells is available. In order to perform this assay, BT474 cells which have been treated with the antibody of interest as described in the preceding two paragraphs are incubated with 9μg/ml HOECHST 33342™ for 2 hr at 37°C, then analyzed on an EPICS ELITE™ flow cytometer (Coulter Corporation) using MODFIT LT™ software (Verity Software House). Antibodies which induce a change in the percentage of apoptotic cells which is 2 fold or greater (and preferably 3 fold or greater) than untreated cells (up to 100% apoptotic cells) may be selected as pro-apoptotic antibodies using this assay. See WO98/17797 for assays for screening for HER2 antibodies which induce apoptosis, such as 7C2 and 7F3.

To screen for antibodies which bind to an epitope on HER2 bound by an antibody of interest, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed to assess whether the antibody cross-blocks binding of an antibody, such as 2C4 or Pertuzumab, to HER2. Alternatively, or additionally, epitope mapping can be performed by methods known in the art and/or one can study the antibody-HER2 structure (Franklin *et al. Cancer Cell* 5:317-328 (2004)) to see what domain(s) of HER2 is/are bound by the antibody.

(ix) Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* a small molecule toxin or an enzymatically active toxin of

bacterial, fungal, plant or animal origin, including fragments and/or variants thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (U.S. Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein.

In one preferred embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (*e.g.* about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al. Cancer Research* 52: 127-131 (1992)) to generate a maytansinoid-antibody immunoconjugate.

Another immunoconjugate of interest comprises a HER2 antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)). See, also, US Patent Nos. 5,714,586; 5,712,374; 5,264,586; and 5,773,001 expressly incorporated herein by reference.

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

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

A variety of radioactive isotopes are available for the production of radioconjugated HER2 antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used.

Alternatively, a fusion protein comprising the HER2 antibody and cytotoxic agent may be made, *e.g.* by recombinant techniques or peptide synthesis.

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

(x) Other antibody modifications

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may 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, Oslo, A., Ed., (1980).

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

WO00/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof. Preferably, the antibody with improved ADCC comprises substitutions at positions 298, 333, and/or 334 of the Fc region. Preferably the altered Fc region is a human IgG1 Fc region comprising or consisting of substitutions at one, two or three of these positions.

Antibodies with altered C1q binding and/or complement dependent cytotoxicity (CDC) are described in WO99/51642, US Patent No. 6,194,551B1, US Patent No. 6,242,195B1, US Patent No. 6,528,624B1 and US Patent No. 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333 and/or 334 of the Fc region thereof.

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 Patent 5,739,277, 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. Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO00/42072 (Presta, L.).

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US Appln No. US2002/0004587 A1, Miller *et al.*).

The HER2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.* 81(19)1484 (1989).

(ix) Exemplary Antibodies

Exemplary antibodies which can be formulated according to the present invention include, but are not limited to the following:

anti-ErbB antibodies, including anti-HER2 antibodies, such as those described in more detail herein;

antibodies that bind to a B-cell surface marker, such as CD19, CD20 (for example Rituximab (RITUXAN®) and humanized 2H7), CD22, CD40 or BR3;

antibodies that bind to IgE, including Omalizumab (XOLAIR®) commercially available from Genentech, E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies. See, also, Presta *et al.*, *J. Immunol.* 151:2623-2632 (1993); International Publication No. WO 95/19181; US Patent No. 5,714,338, issued February 3, 1998; US Patent No. 5,091,313, issued February 25, 1992; WO 93/04173 published March 4, 1993; WO 99/01556 published January 14, 1999; and US Patent No. 5,714,338;

antibodies that bind to vascular endothelial growth factor (VEGF) or a receptor thereof, including Bevacizumab (AVASTIN™), commercially available from Genentech, and Ranibizumab (LUCENTIS™);

anti-IL-8 antibodies (St John *et al.*, *Chest*, 103:932 (1993), and International Publication No. WO 95/23865);

anti-PSCA antibodies (WO01/40309);

anti-CD40 antibodies, including S2C6 and humanized variants thereof (WO00/75348);

anti-CD11a antibodies, including efalizumab (RAPTIVA®) (US Patent No. 5,622,700, WO 98/23761, Steppe *et al.*, *Transplant Intl.* 4:3-7 (1991), and Hourmant *et al.*, *Transplantation* 58:377-380 (1994)); anti-CD18 antibodies (US Patent No. 5,622,700, issued April 22, 1997, or as in WO 97/26912, published July 31, 1997);

anti-Apo-2 receptor antibody (WO 98/51793 published November 19, 1998);

anti-TNF-alpha antibodies including cA2 (REMICADE®), CDP571 and MAK-195 (See, US Patent No. 5,672,347 issued September 30, 1997, Lorenz *et al. J. Immunol.* 156(4):1646-1653 (1996), and Dhainaut *et al. Crit. Care Med.* 23(9):1461-1469 (1995));

anti-Tissue Factor (TF) (European Patent No. 0 420 937 B1 granted November 9, 1994);

anti-human $\alpha 4\beta_7$ integrin (WO 98/06248 published February 19, 1998);
anti-EGFR antibodies, including chimerized or humanized 225 antibody as in WO 96/40210 published December 19, 1996;
anti-CD3 antibodies, such as OKT3 (US Patent No. 4,515,893 issued May 7, 1985);
anti-CD25 or anti-tac antibodies such as CHI-621 (SIMULECT®) and (ZENAPAX®) (See US Patent No. 5,693,762 issued December 2, 1997);
anti-CD4 antibodies such as the cM-7412 antibody (Choy *et al. Arthritis Rheum* 39(1):52-56 (1996));
anti-CD52 antibodies such as CAMPATH-1H (Riechmann *et al. Nature* 332:323-337 (1988);
anti-Fc receptor antibodies such as the M22 antibody directed against FcγRI as in Graziano *et al. J. Immunol.* 155(10):4996-5002 (1995);
anti-carcinoembryonic antigen (CEA) antibodies such as hMN-14 (Sharkey *et al. Cancer Res.* 55(23Suppl): 5935s-5945s (1995);
antibodies directed against breast epithelial cells including huBrE-3, hu-Mc 3 and CHL6 (Ceriani *et al. Cancer Res.* 55(23): 5852s-5856s (1995); and Richman *et al. Cancer Res.* 55(23 Supp): 5916s-5920s (1995));
antibodies that bind to colon carcinoma cells such as C242 (Litton *et al. Eur J. Immunol.* 26(1):1-9 (1996));
anti-CD38 antibodies, *e.g.* AT 13/5 (Ellis *et al. J. Immunol.* 155(2):925-937 (1995));
anti-CD33 antibodies such as Hu M195 (Jurcic *et al. Cancer Res* 55(23 Suppl):5908s-5910s (1995) and CMA-676 or CDP771;
anti-CD22 antibodies such as LL2 or LymphoCide (Juweid *et al. Cancer Res* 55(23 Suppl):5899s-5907s (1995);
anti-EpCAM antibodies such as 17-1A (PANOREX®);
anti-GpIIb/IIIa antibodies such as abciximab or c7E3 Fab (REOPRO®);
anti-RSV antibodies such as MEDI-493 (SYNAGIS®);
anti-CMV antibodies such as PROTOVIR®;
anti-HIV antibodies such as PRO542;
anti-hepatitis antibodies such as the anti-Hep B antibody OSTAVIR®;
anti-CA 125 antibody OvaRex;
anti-idiotypic GD3 epitope antibody BEC2;
anti-$\alpha v\beta 3$ antibody VITAXIN®;
anti-human renal cell carcinoma antibody such as ch-G250; ING-1;
anti-human 17-1A antibody (3622W94);
anti-human colorectal tumor antibody (A33);
anti-human melanoma antibody R24 directed against GD3 ganglioside;
anti-human squamous-cell carcinoma (SF-25); and
anti-human leukocyte antigen (HLA) antibodies such as Smart ID10 and the anti-HLA DR antibody Oncolym (Lym-1).

(xi) Antibody variant compositions

The present invention, in at least one aspect, concerns formulations comprising a composition which comprises a mixture of a main species antibody and one or more variants thereof. Where the main species antibody binds HER2, preferably the HER2 antibody (either or both of the main species HER2 antibody and antibody variant thereof) is one which binds to Domain II of HER2, inhibits HER dimerization more effectively

than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of the main species antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

In one embodiment, the formulated HER2 antibody composition comprises a mixture of the main species HER2 antibody and an amino acid sequence variant thereof comprising an amino-terminal leader extension. Preferably, the amino-terminal leader extension is on a light chain of the antibody variant (*e.g.* on one or two light chains of the antibody variant). The main species HER2 antibody or the antibody variant may be an full length antibody or antibody fragment (*e.g.* Fab of F(ab')2 fragments), but preferably both are full length antibodies. The antibody variant herein may comprise an amino-terminal leader extension on any one or more of the heavy or light chains thereof. Preferably, the amino-terminal leader extension is on one or two light chains of the antibody. The amino-terminal leader extension preferably comprises or consists of VHS-. Presence of the amino-terminal leader extension in the composition can be detected by various analytical techniques including, but not limited to, N-terminal sequence analysis, assay for charge heterogeneity (for instance, cation exchange chromatography or capillary zone electrophoresis), mass spectrometry, etc. The amount of the antibody variant in the composition generally ranges from an amount that constitutes the detection limit of any assay (preferably N-terminal sequence analysis) used to detect the variant to an amount less than the amount of the main species antibody. Generally, about 20% or less (*e.g.* from about 1% to about 15%, for instance from 5% to about 15%) of the antibody molecules in the composition comprise an amino-terminal leader extension. Such percentage amounts are preferably determined using quantitative N-terminal sequence analysis or cation exchange analysis (preferably using a high-resolution, weak cation-exchange column, such as a PROPAC WCX-10™ cation exchange column). Aside from the amino-terminal leader extension variant, further amino acid sequence alterations of the main species antibody and/or variant are contemplated, including but not limited to an antibody comprising a C-terminal lysine residue on one or both heavy chains thereof, a deamidated antibody variant, etc.

Moreover, the main species antibody or variant may further comprise glycosylation variations, non-limiting examples of which include HER2 antibody comprising a G1 or G2 oligosaccharide structure attached to the Fc region thereof, HER2 antibody comprising a carbohydrate moiety attached to a light chain thereof (*e.g.* one or two carbohydrate moieties attached to one or two light chains of the antibody), HER2 antibody comprising a non-glycosylated heavy chain.

III. Preparation of the Formulation

The present invention provides, in a first aspect, a stable pharmaceutical formulation comprising a monoclonal antibody, preferably a full length human or humanized IgG1 antibody, in histidine-acetate buffer, pH 5.5 to 6.5, preferably pH 5.8 to 6.2. However, the antibody in the formulation may be an antibody fragment comprising an antigen-binding region, such as a Fab or F(ab')2 fragment.

In another embodiment, the invention concerns a pharmaceutical formulation comprising, or consisting essentially of, a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL; histidine-acetate buffer, pH 5.5 to 6.5; saccharide selected from the group

consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM; and polysorbate 20 in an amount from about 0.01% to about 0.1%.

In yet a further embodiment, the invention provides a pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide and a surfactant. For example, the formulation may comprise Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5

In another aspect, the invention provides a pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant. Such a formulation may, for example, comprise, Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

The formulation is especially useful for antibodies that are susceptible to deamidation and/or aggregation and/or fragmentation, in that the buffer retards deamidation and/or aggregation and/or fragmentation of the antibody formulated therein. In addition, unlike other histidine buffers prepared using HCl, the histidine-acetate buffer lacks the chloride ion which was found to be beneficial herein in that this buffer when combined with saccharide had the same protective effect on antibody as polysorbate 20, and was stable and compatible with storage in stainless steel tanks. Thus, in addition to the formulation *per se* comprising the antibody susceptible to deamidation, aggregation and/or fragmentation, the invention provides a method for reducing deamidation, aggregation and/or fragmentation of a therapeutic monoclonal antibody (for example, relative to a composition at a different pH or in a different buffer), comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5. In this embodiment, one may determine or measure deamidation, aggregation and/or fragmentation before and after the antibody is formulated, with the formulated antibody demonstrating acceptable deamidation, aggregation and/or fragmentation in the formulation and upon storage thereof.

The antibody in the formulation may bind an antigen including but not limited to: HER2, CD20, IgE, DR5, BR3 and VEGF.

Where the formulated antibody binds HER2, it preferably is one which binds to Domain II of HER2, inhibits HER dimerization more effectively than Trastuzumab, and/or binds to a heterodimeric binding site of HER2. The preferred embodiment herein of a formulated HER2 antibody is one comprising the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, and most preferably comprising the light chain and heavy chain amino acid sequences in SEQ ID Nos. 15 and 16 (Pertuzumab).

Examples of CD20 antibodies which can be formulated herein include: "C2B8" which is now called "Rituximab" ("RITUXAN®") commercially available from Genentech (see also US Patent No. 5,736,137, expressly incorporated herein by reference): the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" ZEVALIN® commercially available from Biogen-Idec (see also US Patent No. 5,736,137, expressly incorporated herein by reference); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "131I-B1" antibody (iodine I131 tositumomab, BEXXAR™) (US Patent No. 5,595,721, expressly incorporated herein by reference): murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework patched" or humanized 1F5 (WO03/002607, Leung, S.); ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (Clark *et al. PNAS* 82: 1766-1770 (1985); US Patent No. 5,500,362, expressly incorporated herein by reference); humanized

2H7: huMax-CD20 (WO 04/035607, Genmab, Denmark); AME-133 (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: Leukocyte Typing III (McMichael, Ed., p. 440, Oxford University Press (1987)).

In the preferred embodiment of a formulated CD20 antibody, the CD20 antibody is a humanized 2H7 antibody. Prefered humanized 2H7 antibodies herein are 2H7v16 and 2H7v511. The humanized 2H7v16 may be an intact antibody or antibody fragment comprising the variable light and variable heavy sequences in Figs. 18A-B (SEQ ID Nos. 26 and 29). Where the humanized 2H7v16 antibody is a full length antibody, preferably it comprises the light and heavy chain amino acid sequences with SEQ ID Nos. 63 and 65.

Where the antibody binds VEGF, it preferably comprises the variable domain sequences as depicted in Fig. 19. The most preferred anti-VEGF antibody is full length humanized IgG1antibody, Bevacizumab (AVASTIN™), commercially available from Genentech.

Where the formulated antibody binds IgE, it is preferably selected from the group consisting of: E25, Omalizumab (XOLAIR®) commercially available from Genentech (see also Figs. 17A-B), E26 (Figs. 17A-B herein), HAE1 (Figs. 17A-B herein), IgE antibody with an amino acid substitution at position 265 of an Fc region thereof (US 2004/0191244 A1), Hu-901 (Figs. 17A-B herein), an IgE antibody as in WO2004/070011, or an antibody (including antibody fragments and full length antibodies) comprising the variable domains of any of those IgE antibodies.

Where the antibody binds to a receptor in the tumor necrosis factor (TNF) superfamily or to a death receptor, it preferably binds to DR5, and preferably is an agonist antibody. Publications in this area include Sheridan *et al.*, *Science*, 277:818-821 (1997), Pan *et al.*, *Science*, 277:815-818 (1997), WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998; Screaton *et al.*, *Curr. Biol.*, 7:693-696 (1997); Walczak *et al.*, *EMBO J.*, 16:5386-5387 (1997); Wu *et al.*, *Nature Genetics*, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999; US 2002/0072091 published August 13, 2002; US 2002/0098550 published December 7, 2001; US 6,313,269 issued December 6, 2001; US 2001/0010924 published August 2, 2001; US 2003/01255540 published July 3, 2003; US 2002/0160446 published October 31, 2002, US 2002/0048785 published April 25, 2002; US 6,342,369 issued February, 2002; US 6,569,642 issued May 27, 2003, US 6,072,047 issued June 6, 2000, US 6,642,358 issued November 4, 2003; US 6,743,625 issued June 1, 2004. The most preferred DR5 antibody is Apomab.

Each of the formulations noted above comprises a buffer, preferably a histidine buffer, and most preferably a histidine-acetate buffer with a pH of 5.5 to 6.5, preferably 5.8 to 6.2, for example approximately 6.0. The concentration of the buffer is dictated, at least in part, by the desired pH. Exemplary concentrations for the buffer are in the range from about 1mM to about 200mM, preferably from about 10mM to about 40mM, most preferably about 20mM.

The antibody concentration in the formulation is preferably in the range from about 10mg/mL to about 250mg/mL. The antibody concentration may be determined based on the intended use and mode of administration of the formulation. For example, where the formulation is for IV administration (*e.g.* a HER2

antibody), the antibody concentration in the formulation is preferably from about 20mg/mL to about 40mg/mL. In the exemplified Pertuzumab formulation intended for intravenous (IV) administration, the antibody concentration was from about 20mg/mL to about 40mg/mL, most preferably about 30mg/mL.

Where the antibody is for SQ or IM administration (*e.g.* for an anti-IgE antibody) higher concentrations of the antibody may be desired. Such substantially high antibody concentrations may be from about 50mg/mL to about 250mg/mL, or from about 80mg/mL to about 250mg/mL, or from about 100mg/mL to about 200mg/mL.

Where the formulation comprises a DR5 antibody, such as Apomab, exemplary antibody concentrations are from about 10mg/mL to about 30mg/mL, for example about 20mg/mL DR5 antibody; such formulation being useful for intravenous administration.

The formulation for administration is preferably an aqueous formulation (not lyophilized) and has not been subjected to prior lyophilization. While the formulation may be lyophilized, preferably it is not. However, freezing of the aqueous formulation, without simultaneous drying that occurs during freeze-drying, is specifically contemplated herein, facilitating longer term storage thereof, for instance in a stainless steel tank.

The formulation preferably further comprises a saccharide, most preferably a disaccharide, such as trehalose or sucrose. The saccharide is generally included in an amount which reduces soluble aggregate formation, such as that which occurs upon freeze/thaw. Exemplary saccharide concentrations are in the range from about 10mM to about 1M, for example from about 60mM to about 250mM, and most preferably about 120mM for a HER2 antibody formulation, and about 240mM for a DR5 antibody formulation.

While it was found herein that a formulation comprising histidine-acetate buffer and saccharide was stable, the formulation optionally further comprises surfactant, such as polysorbate, most preferably polysorbate 20. The surfactant is generally included in an amount which reduces insoluble aggregate formation (such as that which occurs upon shaking or shipping). The surfactant concentration is preferably from about 0.0001% to about 1.0%, most preferably from about 0.01% to about 0.1%, for example about 0.02%.

Optionally, the formulation does not contain a tonicifying amount of a salt such as sodium chloride.

The formulation is generally sterile, and this can be achieved according to the procedures known to the skilled person for generating sterile pharmaceutical formulations suitable for administration to human subjects, including filtration through sterile filtration membranes, prior to, or following, preparation of the formulation.

Moreover, the formulation is desirably one which has been demonstrated to be stable upon storage. Various stability assays are available to the skilled practitioner for confirming the stability of the formulation. For example, the formulation may be one which is found to be stable upon storage: at about 40°C for at least 4 weeks; at about 5°C or about 15°C for at least 3 months or at least 1 year; and/or about -20°C for at least 3 months. Stability can be tested by evaluating physical stability, chemical stability, and/or biological activity of the antibody in the formulation around the time of formulation as well as following storage at the noted temperatures. Physical and/or stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluting biological activity or antigen binding function of the antibody; etc. Instability may result in aggregation, deamidation (*e.g.* Asn deamidation), oxidation (*e.g.* Met oxidation),

isomerization (*e.g.* Asp isomeriation), clipping/hydrolysis/fragmentation (*e.g.* hinge region fragmentation), succinimide formation, unpaired cysteine(s), N-terminal extension, C-terminal processing, glycosylation differences, etc. Biological activity or antigen binding function can be evaluated using various techniques available to the skilled practitioner.

As noted above, freezing of the formulation is specifically contemplated herein. Hence, the formulation can be tested for stability upon freezing and thawing.

According, the invention also provides a method of making a pharmaceutical formulation comprising preparing the formulation as described herein, and evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

In the preferred embodiment, the formulation is provided inside a vial with a stopper pierceable by a syringe, preferably in aqueous form. The vial is desirably stored at about 2-8°C until it is administered to a subject in need thereof. The vial may for example be a 20cc vial (for example for a 420mg dose) or 50cc vial (for example for a 1050mg dose). For a DR5 antibody, such as Apomab, the formulation may be provided in a 5cc glass vial (*e.g.* 5.5ml fill).

In another embodiment, the formulation is provided inside a stainless steel tank. The formulation in the stainless steel tank is optionally frozen and not freeze-dried.

One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (*e.g.* Zn-protein complexes); biodegradable polymers such as polyesters; preservatives; and/or salt-forming counterions such as sodium.

IV. Treatment with the Antibody Formulation

In one embodiment, the invention provides a method of treating a disease or disorder in a subject comprising administering the formulation described herein to a subject in an amount effective to treat the disease or disorder.

Where the antibody in the formulation binds to HER2, it is preferably used to treat cancer. The cancer will generally comprise HER2-expressing cells, such that the HER2 antibody herein is able to bind to the cancer cells. Thus, the invention in this embodiment concerns a method for treating HER2-expressing cancer in a subject, comprising administering the HER2 antibody pharmaceutical formulation to the subject in an amount effective to treat the cancer. Various cancers that can be treated with the composition are listed in the definitions section above.

It is also contemplated that the HER2 antibody formulation may be used to treat various non-malignant diseases or disorders, such a include autoimmune disease (*e.g.* psoriasis); endometriosis; scleroderma; restenosis; polyps such as colon polyps, nasal polyps or gastrointestinal polyps; fibroadenoma; respiratory disease (see definition above); cholecystitis; neurofibromatosis; polycystic kidney disease; inflammatory diseases; skin disorders including psoriasis and dermatitis; vascular disease (see definition above); conditions involving abnormal proliferation of vascular epithelial cells; gastrointestinal ulcers; Menetrier's disease, secreting

adenomas or protein loss syndrome; renal disorders; angiogenic disorders; ocular disease such as age related macular degeneration, presumed ocular histoplasmosis syndrome, retinal neovascularization from proliferative diabetic retinopathy, retinal vascularization, diabetic retinopathy, or age related macular degeneration; bone associated pathologies such as osteoarthritis, rickets and osteoporosis; damage following a cerebral ischemic event; fibrotic or edemia diseases such as hepatic cirrhosis, lung fibrosis, carcoidosis, throiditis, hyperviscosity syndrome systemic, Osler Weber-Rendu disease, chronic occlusive pulmonary disease, or edema following burns, trauma, radiation, stroke, hypoxia or ischemia; hypersensitivity reaction of the skin; diabetic retinopathy and diabetic nephropathy; Guillain-Barre syndrome; graft versus host disease or transplant rejection; Paget's disease; bone or joint inflammation; photoaging (*e.g.* caused by UV radiation of human skin); benign prostatic hypertrophy; certain microbial infections including microbial pathogens selected from adenovirus, hantaviruses, Borrelia burgdorferi, Yersinia spp. and Bordetella pertussis; thrombus caused by platelet aggregation; reproductive conditions such as endometriosis, ovarian hyperstimulation syndrome, preeclampsia, dysfunctional uterine bleeding, or menometrorrhagia; synovitis; atheroma; acute and chronic nephropathies (including proliferative glomerulonephritis and diabetes-induced renal disease); eczema; hypertrophic scar formation; endotoxic shock and fungal infection; familial adenomatosis polyposis; neurodedenerative diseases (*e.g.* Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration); myelodysplastic syndromes; aplastic anemia; ischemic injury; fibrosis of the lung, kidney or liver; T-cell mediated hypersensitivity disease; infantile hypertrophic pyloric stenosis; urinary obstructive syndrome; psoriatic arthritis; and Hasimoto's thyroiditis. Preferred non-malignant indications for therapy herein include psoriasis, endometriosis, scleroderma, vascular disease (*e.g.* restenosis, artherosclerosis, coronary artery disease, or hypertension), colon polyps, fibroadenoma or respiratory disease (*e.g.* asthma, chronic bronchitis, bronchiectasis or cystic fibrosis).

Where the antibody in the formulation binds to a B-cell surface marker such as CD20 or BR3, the formulation may be used to treat a B-cell malignancy, such as NHL or CLL, an autoimmune disease, graft rejection, or to block an immune response to a foreign antigen, such as an antibody, a toxin, a gene therapy viral vector, a graft, an infectious agent, or an alloantigen (see WO 01/03734, Grillo-Lopez *et al.*).

Where the antibody in the formulation is an IgE antibody, it may be used to treat an IgE-mediated disorder (USSN 2004/0197324 A1, Liu and Shire), such as allergic asthma, allergic rhinitis, atopic dermatitis, allergic gastroenteropathy, hypersensitivity, eczema, urticaria, allergic bronchopulmonary aspergillosis, parasitic disease, hyper-IgE syndrome, ataxia-telangiectasia, Wiskott-Aldrich syndrome, thymic alymphoplasia, IgE myeloma, and graft-versus-host reaction.

Antibodies that bind to a receptor in the TNF superfamily (for instance which bind to DR5), or which bind to VEGF (or a receptor thereof), may be used to treat cancer, various forms of which are described in the definitions section above. Preferably, the cancer treated with a DR5 antibody formulation is a solid tumor or NHL.

Where the indication is cancer, the patient may be treated with a combination of the antibody formulation, and a chemotherapeutic agent. The combined administration includes coadministration or concurrent administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Thus, the chemotherapeutic agent may be administered prior to,

or following, administration of the composition. In this embodiment, the timing between at least one administration of the chemotherapeutic agent and at least one administration of the composition is preferably approximately 1 month or less, and most preferably approximately 2 weeks or less. Alternatively, the chemotherapeutic agent and the composition are administered concurrently to the patient, in a single formulation or separate formulations.

Treatment with the formulation will result in an improvement in the signs or symptoms of cancer or disease. For instance, where the disease being treated is cancer, such therapy may result in an improvement in survival (overall survival and/or progression free survival) and/or may result in an objective clinical response (partial or complete). Moreover, treatment with the combination of the chemotherapeutic agent and the antibody formulation may result in a synergistic, or greater than additive, therapeutic benefit to the patient.

Preferably, the antibody in the formulation administered is a naked antibody. However, the antibody administered may be conjugated with a cytotoxic agent. Preferably, the immunoconjugate and/or antigen to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with nucleic acid in the cancer cell. Examples of such cytotoxic agents include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The formulation is administered to a human patient in accord with known methods, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous, intramuscular or subcutaneous administration of antibody composition is preferred, with intravenous administration being most preferred.

For subcutaneous delivery, the formulation may be administered via syringe: injection device (*e.g.* the INJECT-EASE™ and GENJECT™ device); injector pen (such as the GENPEN™); needleless device (*e.g.* MEDIJECTOR™ and BIOJECTOR™); or subcutaneous patch delivery system.

For the prevention or treatment of disease, the appropriate dosage of the antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody 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 antibody 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 50 mg/kg (*e.g.* 0.1-20mg/kg) of HER2 or DR5 antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. The dosage of the antibody will generally be in the range from about 0.05mg/kg to about 10mg/kg. If a chemotherapeutic agent is administered, it is usually administered at dosages known therefor, or optionally lowered due to combined action of the drugs or negative side effects attributable to administration of the chemotherapeutic agent. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992).

Other therapeutic regimens may be combined with the antibody including, but not limited to: a second (third, fourth, etc) chemotherapeutic agent(s) *(i.e.* "cocktails" of different chemotherapeutic agents); another

monoclonal antibody;a growth inhibitory agent; a cytotoxic agent; a chemotherapeutic agent; EGFR-targeted drug; tyrosine kinase inhibitor; anti-angiogenic agent; and/or cytokine; etc.

In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

5

V. Articles of Manufacture

In another embodiment of the invention, an article of manufacture is provided which contains the pharmaceutical formulation of the present invention and provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (*e.g.* dual chamber 10 vials), syringes (such as dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including 15 other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use as noted in the previous section.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature and patent citations are incorporated herein by reference.

20

EXAMPLES

Stable Pertuzumab Liquid Formulations

These examples describe the development and stability testing of stable liquid formulations comprising Pertuzumab at protein concentrations in the range from about 10 mg/mL – 180 mg/mL. The selected 25 formulations had low turbidity, and were physically and chemically stable. A chloride ion was removed from the formulation to reduce the risk of corrosion. The formulation was isotonic, and suitable for subcutaneous or intramuscular delivery. Insoluble aggregate formation upon agitation stress was prevented using histidine-acetate and sucrose formulation, without the need to include polysorbate 20.

30 **Analytical Methods**

Color, Appearance and Clarity (CAC)

The color, appearance, and clarity of the samples were determined by visual inspection of vials against a white and black background under white fluorescence light at room temperature.

35 **UV Concentration Measurements**

The liquid product aliquot was first diluted with formulation buffer so that the A_{max} near 278 nm is within 0.5-1.0 absorbance unit. The UV absorbance of the diluted samples was measured in a quartz cuvette with 1 cm path length on an HP 8453 spectrophotometer. Absorbance was measured at 278 nm and 320 nm. The absorbance from 320 nm is used to correct background light scattering due to larger aggregates, bubbles and

particles. The measurements were blanked against the formulation buffer. The protein concentration was determined using the absorptivity of 1.50 $(mg/mL)^{-1}cm^{-1}$.

pH measurements

The pH was measured at room temperature using a RADIOMETER COPENHAGEN PHM82™ pH meter. The probe used was a combined glass/reference electrode with radiometer connector (Sigma, Cat# E-5759). Standard solutions of pH 4.01 and pH 7.00 (EM Science) were used for calibration of the pH meter.

Ion-Exchange Chromatography (IEX)

Cation exchange chromatography was employed to measure changes in charge variants. This assay utilizes a DIONEX PROPAC WCX-10™ column on an HP 1100™ HPLC system. Samples were diluted to 1 mg/mL with the mobile phase A containing 20 mM MES at pH 6.0. 50 mL of diluted samples were then loaded on the column that was kept at ambient temperature. The peaks were eluted with a shallow NaCl gradient using mobile B containing 20 mM MES, 250 mM NaCl, pH 6.0. The eluent was monitored at 280 nm. The data were analyzed using HP CHEMSTATION™ software (Rev A08.03).

Capillary Zone Electrophoresis (CZE)

The purity of Fab and $F(ab')_2$ fragments was determined by CZE. This assay was run on a BIORAD BIOFOCUS™ 3000™ capillary electrophoresis system with a BIOCAP XL™ capillary, 50 µm I.D., 44.6 cm total length and 40 cm to the detector.

Size Exclusion Chromatography (SEC)

Size exclusion chromatography was used to quantitate aggregates and fragments. This assay utilizes a TSK G3000 SWXL™, 7.8 x 300 mm column and runs on an HP 1100™ HPLC system. Samples were diluted to 10 mg/mL with the mobile phase and injection volume was 20 µL. The mobile phase was 100 mM K_2HPO_4 at pH 6.8 and the protein was eluted with an isocratic gradient at 0.5 mL/min for 45 minutes. The eluent absorbance was monitored at 280 nm. Integration was done using HP CHEMSTATION™ software (Rev A08.03).

Biological Activity

The biological activity of Pertuzumab was determined by measuring its ability to inhibit proliferation of the human breast cancer cell line MDA-MB-175-VII.

EXAMPLE 1

Pertuzumab Fab and $F(ab')_2$ antibody fragments were formulated at protein concentration of 1.0 mg/mL in the following buffer conditions:

10 mM citrate, 140 mM NaCl, pH 4.0;

10 mM succinate, 140 mM NaCl, pH 5.0;

10 mM succinate, 140 mM NaCl,, pH 6.0;

10 mM histidine, 140 mM NaCl, pH 7.0; and

10 mM glycylglycine, 140 mM NaCl, pH 8.0.

Each formulation was filtered then aliquoted into 3 cc WHEATON™ USP Type I glass vials sealed with TEFLON™ coated gray butyl stoppers. Samples were stored at 40 ± 2 °C. The stability analyses of drug product showed that the Fab and $F(ab')_2$ were most stable between pH 5.0 and 6.0.

Table 2. Effect of pH on degradation of Fab or $F(ab')_2$ stored at 40°C

Formulation pH	Fab		$F(ab')_2$	
	CZE % Main Peak	SEC % Main Peak	CZE % Main Peak	SEC % Main Peak
4.0	74.1	96.7	43.6	89.4
5.0	83.2	96.4	65.4	94.0
6.0	82.9	96.2	69.0	92.3
7.0	83.9	96.4	62.3	91.3
8.0	72.7	96.4	49.2	89.8

EXAMPLE 2

Pertuzumab was formulated into 20 mM histidine-acetate buffer with 120 mM sucrose and 0.02 % polysorbate 20. The pHs of formulations were adjusted with acetic acid to final pH between 5.0 and 7.0. The protein concentration was 30 mg/mL. Each formulation was filled into 3 cc USP Type I glass vials and stored at 40°C for stability analysis. The results showed that Pertuzumab was most stable around pH 6.0.

Table 3. Effect of pH on degradation of Pertuzumab stored at 40°C

Formulation pH	Temperature (°C)	Storage Time (wks)	SEC % Monomer	IEX % Main Peak
5.0	40	2	99.4	57.4
5.5	40	2	99.4	59.2
6.0	40	2	99.4	60.6
6.5	40	2	99.3	60.5
7.0	40	2	99.1	54.0
5.0	40	4	97.3	48.1
5.5	40	4	99.1	50.5
6.0	40	4	99.1	53.3
6.5	40	4	99.0	52.3
7.0	40	4	98.6	42.3

EXAMPLE 3

Pertuzumab formulations at protein concentration of 100 mg/mL were prepared in the following excipients:

(1) 10 mM histidine-HCl, 240 mM sucrose, 0.02% polysorbate 20, pH 6.0;

(2) 10 mM histidine-acetate, 240 mM sucrose, 0.02% polysorbate 20, pH 6.0;
(3) 10 mM histidine-phosphate, 240mM sucrose, 0.02% polysorbate 20, pH 6.0;
(4) 10 mM histidine-sulfate, 240 mM sucrose, 0.02% polysorbate 20 at pH 6.0.

Each formulation was filled into 3 cc FORMA VITRUM™ USP Type I glass vial sealed with FLUROTEC™ faced butyl rubber stoppers. Samples were stored at 30°C and 40°C and stability was evaluated for quality (CAC) and purity (SEC, IEC). The stability results showed that Pertuzumab in histidine-phosphate buffer degraded much faster than in other histidine buffers upon storage at 40°C (Fig. 8 and Fig. 9).

EXAMPLE 4

Pertuzumab was concentrated by ultrafiltration/diafiltration to various concentrations in the following buffers:
(1) 20 mM histidine-acetate, pH 6.0;
(2) 10 mM histidine-HCl, pH 6.0, and
(3) 10 mM histidine-sulfate, pH 6.0.

The turbidity of each formulation was measured before the filtration. The results, as shown in Fig. 10, demonstrated that Pertuzumab samples formulated in histidine-acetate and histidine-HCl had less amounts of insoluble aggregates than those in histidine-sulfate buffer.

EXAMPLE 5

Pertuzumab was formulated at 30 mg/mL in 20 mM histidine-acetate, 120 mM sucrose, 0.02 % polysorbate 20, pH 6.0. Pertuzmab was filled in 316L and HASTELLOY™ stainless steel miniature tanks. All samples were stored at -20°C and 5°C and evaluated for quality (CAC), purity (SEC, IEC) and strength (UV-Vis). The stability analyses showed that Pertuzumab was stable in this formulation upon storage at –20°C and 5°C for at least 3 months. The chloride free formulation is compatible with 316L and HASTELLOY™ stainless steel tank.

Table 4. Stability of Pertuzumab in Stainless Steel Tanks

Tanks	Temp (°C)	Time (Months)	CAC	UV Spec. (mg/mL)	SEC (% monomer)	IEC (% main peak)
		0	Pass[a]	29.0	99.8	67.9
316L	- 20	3	Pass	28.9	99.7	66.8
	5	3	Pass	28.7	99.7	66.8

HASTELLOY™	- 20	3	Pass	29.1	99.7	66.8
	5	3	Pass	28.8	99.7	67.7

[a] Pass for Color, Appearance and Clarity: Clear to slightly opalescent, colorless to pale yellow solution.

EXAMPLE 6

Pertuzumab was formulated using tangential flow filtration (TFF). The final formulation contains 20 mM histidine-acetate, 120 mM sucrose, 0.02 % polysorbate 20, pH 6.0 at protein concentration of 30 mg/mL. Samples were filled into a 20 Ml FORMA VITRUM™ USP Type I glass vial, capped with the 20 mm FLUROTEC™ faced butyl rubber stoppers, and sealed with aluminium flip-top caps. All samples were stored at –70°C. 5°C. 15°C, and stability was evaluated for quality (CAC), purity (SEC, IEC), strength (UV-Vis), and potency (Bioassay). The results showed that Pertuzumab is stable in this formulation upon storage at 5°C and 15°C for at least 3 months.

Table 5. Stability of Pertuzumab in glass vials

Temp (oC)	Time (Months)	CAC	UV Spec. (mg/mL)	SEC (% monomer)	IEC (% main peak)	Bioassay (% specific activity)
	0	Pass	29.2	99.8	64.1	83
-70	1	Pass	29.7	99.8	65.2	92
	3	Pass	30.7	99.8	67.0	93
5	3	Pass	30.4	99.7	67.2	90
15	1	Pass	29.7	99.7	64.4	78
	3	Pass	30.4	99.7	65.5	93

EXAMPLE 7

Pertuzumab was formulated at 100 mg/mL in the following buffer conditions:

(1) 10 mM histidine-HCl, pH 6.0;

(2) 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(3) 20 mM succinate at pH 6.0; and

(4) 20 mM succinate, 240 mM sucrose at pH 6.0.

Each formulation was added with different concentration of polysorbate 20. All samples were filled into 3 cc USP Type I glass vials and were agitated horizontally at 70 rpm at room temperature for up to 7 days. The stability of each sample was evaluated at 7 day time point for turbidity. The results demonstrated that the use of polysorbate 20 in the final formulation effectively prevented formation of insoluble aggregates. See Fig. 11.

EXAMPLE 8

Pertuzumab was prepared in the following formulations:

(1) 25 mg/mL Pertuzumab, 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(2) 50 mg/mL Pertuzumab, 10 mM histidine-HCl, 240 mM sucrose, pH 6.0;

(3) 60 mg/mL Pertuzumab, 20 mM histidine-acetate, 120 mM sucrose, pH 6.0.

Various amounts of polysorbate 20 were added to each formulation. All samples were filled into 3 cc USP Type I glass vials, and agitated horizontally at 70 rpm at room temperature for up to 7 days. The physical stability of each sample was evaluated at 7 day time point for turbidity. The results demonstrated that the use of polysorbate 20 in histidine-HCl and sucrose formulation effectively prevented formation of insoluble particulates. The formulation containing histidine-acetate and sucrose appeared to have the same protective effect on protein as polysorbate 20. See Fig. 12.

EXAMPLE 9

Pertuzumab was formulated as follows:

(1) 100 mg/mL protein, 10 mM histidine-HCl, pH 6.0;

(2) 100 mg/mL protein, 20 mM succinate, pH 6.0;

(3) 60 mg/mL protein, 20 mM histidine-acetate, pH 6.0.

Each formulation was mixed with different amounts of sucrose. All samples were sterilely filled into 3 cc USP Type I glass vials. They were then frozen at –70°C and thawed at 5°C three times. The physical stability of each sample was determined after the three cycles of freezing and thawing. The results demonstrated that sucrose prevents soluble aggregate formation during the freeze-thawing process. See Fig. 13.

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EXAMPLE 10

The preferred Pertuzumab formulation for therapeutic use consists essentially of 30mg/mL Pertuzumab in 20mM histidine acetate, 120mM sucrose, 0.02% polysorbate 20, at pH 6.0.

Compound	Concentration	Amount/L
Pertuzumab	30 mg/mL	30 g
L-Histidine MW=155.16g/mol	20 mM	3.10 g
Glacial Acetic Acid MW=60.05g/mol Density=1.05g/cm^3	11.6 mM	0.66 mL
Sucrose MW=342.3g/mol	120 mM	41.1 g
Polysorbate 20 Density=1.012g/cm^3	0.02% (w/v)	0.2 mL

MW: Molecular weight

420mg dose vial configuration:
Vial: 20 cc Formal Vitrum Type I glass
Stopper: 20mm DAIKYO GREY™, fluoro-resin laminated
Cap: 20mm flip top aluminum
Fill volume: 14.50 mL
Delivery: 14.0 mL Pertuzumab in normal saline IV bag.

1050mg dose vial configuration:
Vial: 50 cc Formal Vitrum Type I glass
Stopper: 20mm DAIKYO GREY™, fluoro-resin laminated
Cap: 20mm flip top aluminum
Fill volume: 36.0 mL
Delivery: 35.0 mL Pertuzumab in normal saline IV bag.

EXAMPLE 11

This example concerns another Pertuzumab formulation which has been used in Phase I and Phase II clinical trials. The composition consists of 25 mg/ml Pertuzumab, 10 mM Histidine-HCl buffer, 240 mM sucrose, 0.02% Polysorbate 20, pH 6.0.

Ingredient	Concentration
Pertuzumab	25 mg/ml
L-His HCl.H_2O (MW 209.6)	1.12 mg/ml (0.0125 M)
L-His(MW 155.2)	0.72 mgml (0.0099 M)
Sucrose (MW342.3)	82.15 mg/ml (0.240 M)
Polysorbate 20	0.2 mg/ml (0.02 %)

EXAMPLE 12

Cellular apoptosis is mediated by intrinsic and extrinsic pathways. Chemotherapy can cause cell damage and may trigger apoptosis by the intrinsic pathway in response to cellular damage. However, cancer cells often develop resistance to chemotherapy through mutations in the p53 tumor suppressor gene (Ashkenazi A. Targeting Death and Decoy Receptors of the Tumour-Necrosis Factor Superfamily. *Nature Reviews* 2:420-430 (2002)). Death receptors, such as DR4 and DR5, located on the surface of cells trigger apoptosis via the extrinsic pathway that does not involve p53. Agonistic molecules, such as Apo2L, bind to DR4 and DR5 receptors and activate caspases 8 and 10 through Fas-associated death domain. Caspase 8 and 10 then activate caspases 3, 6, and 7 to induce apoptosis. Molecular signaling of death receptors on tumor cells has therapeutic potential for the elimination of cancer cells that are resistant to conventional therapies and molecules, like Apo2L, are currently undergoing clinical evaluation.

"Apomab" is a full-length CHO derived humanized IgG1 constructed with a lamda light chain. It is an agonist antibody against DR5 that has been shown to induce apoptosis of various cancer cell lines. Preclinical studies using a murine tumor implant model have shown that Apomab has similar or improved tumor reduction compared to Apo2L. Apomab is being evaluated as an anti-cancer agent in the indications of advanced solid tumors and Non-Hodgkin's Lymphoma (NHL). The heavy and light chain amino acid sequences of Apomab used in these experiments are shown in Figs. 27 and 28.

Preparation of Antibody Formulations

Recombinantly produced Apomab had very dilute protein concentration and high pH. The material was concentrated to approximately 20 mg/mL and exchanged into 20 mM sodium acetate, pH 5.0 buffer using a Millipore Labscale tangential flow filtration (TFF) system with MILLIPORE PELLICON™ XL, PLCGC10, 50 cm membrane. Apomab samples were formulated into various buffer systems covering pH range from 4.0 to 7.0 using sodium acetate, histidine acetate, and sodium phosphate without trehalose and TWEEN 20® using dialysis with a 10,000 Da molecular weight cut off membrane (Pierce, Inc). Trehalose at 240 mM was added in the last dialysis. After dialysis, 0.02% TWEEN 20™ was added to the formulation and the samples were filtered with 0.22 µm filters (Millipore, Inc.). A 0.5 mL volume of Apomab was filled into sterile 3 cc glass vials (Forma Vitrum, Inc.) and sealed with 13 mm stoppers (Daikyo, Inc). Protein stability was evaluated at -70°C, 5°C, 30°C, and 40°C with storage for up to 3 months.

Stability of Apomab Formulation

For drug product stability testing, Apomab formulated bulk filled into 5 cc FORMA VITRUM® glass vials were formulated. Vials were filled with 5.5 mL of formulated antibody, fitted with 20 mm DAIKYO® stoppers, and stored at -70°C, 5°C, 30°C, and 40°C in the upright position.

For drug substance stability testing, Apomab formulated bulk was sterile filtered through a 0.22 µm filter and 10 mL was filled into autoclaved 20cc 316L stainless steel mini-tanks. The tanks were placed upright at –20°C and 5°C. A 1 mL aliquot was aseptically removed from the mini-tanks at specified time intervals to assess protein quality. The control vials were 1 mL aliquots in 3 cc glass vials stored at -20°C.

Color, Appearance, and Clarity

The clarity, appearance, and color of the samples were visually assessed under white fluorescent light using a light inspection station with black and white background. For analysis of the drug substance, mini-tank samples were transferred to a 3 cc glass vial for inspection.

pH

pH was measured at room temperature with THERMO ORION SURE-FLOW ROSS™ semi-micro pH electrode for measuring buffers or THERMO ORION GLS™ combination micro pH electrode for measuring protein pH screening samples, a Beckman microelectrode probe for Toxicology stability samples. The METERLAB™ pHM240 pH/Ion meter (Radiometer Analytical) was calibrated every day with buffer standards (EM Science) at pH 7 and pH 4.

Concentration

Protein concentration was determined by ultraviolet absorption spectroscopy using an AGILENT 8453™ spectrophotometer. The samples were diluted with appropriate formulation buffer blanks to give an absorbance from 0.5 to 1.0. The instrument was blanked with the diluent solution and the spectrum was scanned from 240 to 500 nm. The absorbance value at 320 nm was subtracted from the absorbance at 279 nm to correct for offset and light scattering. The protein concentrations were calculated by the following equation:

$$\text{Conc. (mg/mL)} = \frac{(A279 - A320) \times \text{dilution factor}}{\text{absorptivity coefficient in } cm^{-1}(mg/mL)^{-1}}$$

The absorptivity coefficient based on sequence was initially determined to be 1.32 $cm^{-1}(mg/mL)^{-1}$ and this value was used for the pH screening studies. A later value of 1.7 $cm^{-1}(mg/mL)^{-1}$ was determined by amino acid analysis and proteolysis methods and this value was used for the stability analysis of Apomab used in Toxicology studies.

Ion-Exchange Chromatography

Ion exchange chromatography was carried out on an 1100 series HPLC (Agilent Technologies, Inc.) equipped with a diode array detector. Chromatography was carried out on a PROPAC WCX-10™ (Dionex) column (4 x 250 mm) at a flow rate of 0.5 mL/min and with column temperature at 40°C. Mobile phase A was

25 mM sodium phosphate, pH 6.5. Mobile phase B was 100 mM sodium chloride in the same buffer as mobile phase A. The column was equilibrated with 100% mobile phase A. For pH screening samples an amount of 20 mg of Apomab was loaded onto the column and the absorbance was monitored at 214 nm. Protein was eluted from the column with the following gradient:

Time (min)	% A	%B
0	100	0
50	0	100
51	100	0
70	100	0

For stability analysis of material used in the Toxicology studies an amount of 30 mg of Apomab was loaded onto the column and the absorbance was monitored at 280 nm. Protein was eluted from the column with the following gradient:

Gradient:	Time (min)	% A	%B
	0	100	0
	40.0	40	60
	41.0	0	100
	45.0	0	100
	45.1	100	0
	60.0	100	0

Size-Exclusion Chromatography

Size exclusion chromatography was carried out on an 1100 series HPLC (Agilent Technologies, Inc.) equipped with a diode array detector. An amount of 50 µg Apomab was loaded onto a TSK Gel 3000SWXL™ (7.8 x 300 mm) column and run at a flow rate of 0.9 mL/min for 20 minutes for pH screening samples and 0.5 mL/min for 30 minutes for Toxicology stability samples with 0.20 M potassium phosphate, 0.25 M potassium chloride, pH 6.2 as a mobile phase. Absorbance was monitored at 280 nm.

Potency

The purpose of the potency bioassay was to measure the ability of Apomab to kill Colo205 cells using ALAMARBLUE™. Colo205 is a colon carcinoma cell line, which expresses both DR5 and DR4 death receptors. This assay incorporates a fluorometric/colorimetric growth indicator based on detection of metabolic activity. ALAMARBLUE™ is a redox dye that is blue and non-fluorescent in oxidized state. The intracellular metabolic reduction converts it into a red color that is also fluorescent. The changes in color and fluorescence are proportional to the metabolic activity and number of living cells. The signal decreased when cells die. Apomab was diluted in medium with anti-Fc and then Colo 205 cells were added to Apomab samples and incubate at 37°C for 48 hours. ALAMARBLUE™ is added for the last 2-3 hours. The plate was read at 530 nm excitation and 590 nm emission to get relative fluorescence units (RFU). The data were analyzed by KALEIDAGRAPH™. A dilution curve of killing was generated.

RESULTS

Formulation pH Screen Study

The effect of pH on antibody stability was studied using Apomab produced from an unamplified stable cell line. For this analysis, Apomab was formulated at 20 mg/mL antibody in 20 mM sodium acetate buffer at pH 4.0, 4.5, 5.0, 5.5; 20 mM histidine acetate buffer at pH 6.0 and 6.5; and 20 mM sodium phosphate buffer at pH 7.0. All of the formulations contained 240 mM trehalose and 0.02% TWEEN 20®. The formulations were stored for up to 3 months at temperatures of -70°C, 5°C, 30°C, and 40°C and protein stability was determined by various analytical assays, including CAC, pH, concentration, SEC and IEC. No significant changes in CAC, pH or protein concentration were observed during storage of the samples.

Analysis of the samples by SEC showed that no significant changes occurred during storage at 5°C and -70°C. However, degradation observed as the formation of antibody fragments and soluble aggregates occurred during storage at 30°C and 40°C (Fig. 20). To compare the formulations, antibody monomer kinetics during storage was monitored and the first-order rate constants were calculated. The obtained pH rate profile for the loss in antibody monomer is shown in Fig. 21. The optimal condition for the stability of antibody monomer was obtained by formulating in histidine acetate buffer at pH 6.0.

Apomab charge heterogeneity was monitored by IEC. No significant changes in the IEC profile occurred during storage at 5°C and -70°C. However, degradation observed as the formation of acidic or basic variants occurred depending on the formulation (Fig. 22). In general, increased basic variants were formed at lower formulation pH and more acidic variants were formed at higher formulation pH. To compare the formulations, IEC main peak kinetics was monitored during storage and the first-order rate constants were calculated. The obtained pH rate profile for the loss in IEC main peak is shown in Fig. 23. The rate constants observed by IEC were approximately 10 fold higher than those from SEC (Fig. 21). Therefore, the loss in IEC main peak was the primary degradation of the antibody that will ultimately limit the product shelf life. Furthermore, as observed by SEC, optimal antibody stability to stabilize IEC main peak was obtained by formulating in histidine acetate buffer at pH 6.0.

Following the analysis of pH screening data described above, an Apomab formulation was selected that comprised 20 mg/mL antibody in 20 mM histidine acetate, 240 mM trehalose, 0.02% polysorbate 20, pH 6.0. For the drug product, the vial configuration consisted of 5.5 mL fill in a 5 cc FORMA VITRUM™ vial with a 20 mM DAIKYO™ West stopper. Apomab was stored in stainless steel tanks.

The stability of Apomab Drug Product was evaluated in the 5cc glass vial configuration described above. Vials were stored at -70°C (controls), 5°C, 30°C, and 40°C. Samples were pulled at specific time intervals and analyzed by the following assays: color, appearance, clarity (CAC), pH, protein concentration, SEC, IEC, and potency. The results from these assays are shown in Table 6 for samples stored at -70°C and 5°C and Table 7 for samples stored at 30°C and 40°C.

Table 6. Stability Data for Apomab Stored at -70°C and 5°C

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ± 0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 - 140%*
NA	T=0	Clear	Colorless	5.9	20.2	99.8	63	94
-70	1 month	Clear	Colorless	6.0	20.5	99.8	63	86
-70	2 month	Clear	Colorless	6.0	20.4	99.7	64	91
-70	3 month	Clear	Colorless	6.0	20.5	99.7	63	83
-70	6 month	Clear	Colorless	6.0	20.4	99.7	64	85
-70	9 month	Clear	Colorless	6.0	20.4	99.8	65	89
-70	12 month	Clear	Colorless	6.0	20.8	99.7	63	107
5	1 month	Clear	Colorless	6.0	20.5	99.7	63	89
5	2 month	Clear	Colorless	6.0	20.4	99.7	64	99
5	3 month	Clear	Colorless	6.0	20.8	99.7	63	84
5	6 month	Clear	Colorless	6.0	20.5	99.7	64	93
5	9 month	Clear	Colorless	6.0	20.6	99.7	64	88
5	12 month	Clear	Colorless	6.0	20.7	99.6	64	106

Table 7. Stability Data for Apomab Stored at 30°C and 40°C

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ± 0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 - 140%*
30	1 month	Clear	Colorless	6.0	20.6	98.2	59	91
30	2 month	Clear	Colorless	6.0	20.3	97.4	54	80
30	3 month	Clear	Colorless	6.0	20.6	97.2	49	74
30	6 month	Clear	Colorless	6.0	20.2	94.1	37	51
30	9 month	Clear	Slightly yellow	6.0	20.4	93.2	31	55
30	12 month	Clear	Slightly yellow	6.0	20.6	91.6	25	59
40	1 month	Clear	Colorless	6.0	20.4	96.6	44	79
40	2 month	Clear	Colorless	6.0	20.0	93.7	31	64
40	3 month	Clear	Slightly yellow	5.9	20.3	91.5	22	53
40	6 month	Clear	Slightly yellow	6.0	20.2	83.9	NT	28
40	9 month	Clear	Yellow	5.9	20.3	76.8	NT	25
40	12 month	Clear	Yellow	5.9	20.5	71.4	NT	31

NT = not quantitated

No change in protein quality was observed after twelve months storage at -70°C and 5°C. For instance, the pH remained at 6.0 ± 0.3. Apomab appeared as a clear and colorless liquid, the protein concentration remained at 20.0 ± 2.0 mg/mL, and % monomer was unchanged. Furthermore, there was no significant change in % IEC main peak and % specific activity determined by the cell-killing potency assay was within the assay precision of 60 % to 140 % specific activity. The results showed that Apomab stored in 5 cc glass vials was stable for at least 12 months at 5°C.

Table 7 shows that changes in protein quality occurred at 30°C and 40°C. SEC showed a decrease in % monomer with a rise primarily in fragment species. Aggregates increase as well at higher temperature, but the rate was much slower. However, the aggregates increase significantly after 6 months at 40°C. IEC % main peak decreased with a corresponding increase in acidic variants. Basic peaks decreased slightly after 2 months at 40°C and 9 months at 30°C. After six months of storage at 40°C, degradation occurred to an extent that IEC main peak could no longer be integrated. The cell killing bioassay showed loss of % specific activity at higher temperature with longer storage time. Protein concentration and pH were unchanged. The solution becomes slightly yellow after 3 months at 40°C and 9 months at 30°C and becomes yellow after 9 months at 40°C.

Drug Substance Stability

Freeze-thaw stability data for drug substance are shown in Table 8.

Table 8. Freeze-Thaw Stability Data for Apomab Filled in Miniature Stainless Steel Tanks

Temp (°C) (Frozen/thaw)	Freeze-Thaw Cycle No.	Clarity	Color	pH	Concentration (mg/mL)	SEC (% Monomer)
Acceptance Criteria:		*Report*	*Report*	*6.0±0.3*	*20.0±2.0*	*≥95%*
Control (unfrozen)	0	Clear	Colorless	6.0	20.9	99.6
-20/25	1	Clear	Colorless	6.0	20.8	99.6
-20/25	2	Clear	Colorless	6.0	20.8	99.6
-20/25	3	Clear	Colorless	6.0	20.9	99.6

Table 9. Stability Data for Apomab Filled in Miniature Stainless Steel Tanks

Temp (°C)	Time Point	Clarity	Color	pH	Concentration (mg/mL)	SEC (% monomer)	IEC (% main peak)	Potency (% Specific Activity)
Acceptance Criteria:		*Report*	*Report*	*6.0 ± 0.3*	*20 ± 2*	*≥ 95%*	*Report*	*60 – 140%*
NA	T=0	Clear	Colorless	5.9	20.0	99.7	63	88
-20	1 month	Clear	Colorless	6.0	20.6	99.7	63	107
-20	3 month	Clear	Colorless	6.0	20.6	99.7	63	82
-20	6 month	Clear	Colorless	6.0	20.3	99.7	64	92
-20	9 month	Clear	Colorless	6.0	20.6	99.7	64	92
-20	12 month	Clear	Colorless	6.0	21.2	99.7	65	94
5	1 month	Clear	Colorless	6.0	20.5	99.7	62	95
5	3 month	Clear	Colorless	6.0	20.7	99.6	62	71
5	6 month	Clear	Colorless	6.0	20.4	99.5	62	84
5	9 month	Clear	Colorless	6.0	20.8	99.4	61	84
5	12 month	Clear	Colorless	6.0	21.3	99.2	59	82

No significant changes in the chemical characteristics of the protein were observed after being frozen at -20°C for at least 15 hours and thawed at ambient temperature three times. For example, Apomab appeared as a clear and colorless liquid, the pH remained at 6.0 ± 0.3, and the SEC monomer peak percentage was unchanged.

5

Apomab stability in stainless steel containers was evaluated at -20°C and 5°C (Table 9).

Samples were aseptically pulled from the mini-tanks at specific intervals and analyzed.

Apomab showed no change in protein quality at 5°C by pH, CAC, protein concentration and % main peak by IEC but lost 0.1% monomer by SEC every 3 months. Decreased potency was observed during storage at 5°C for

3 months. However, the potency of the sample increased again at the 6 and 9 month timepoints. Therefore, the observed potency difference at the 3 month timepoint was attributed to assay variation. Apomab showed no change in protein quality at -20°C by pH, CAC, protein concentration, % monomer by SEC, % main peak by IEC, and no significant change in potency. The stability data show that Apomab is stable for at least 1 year at -20°C and three months at 5°C.

CONCLUSION

Formulation screening studies were performed to select a formulation for Apomab. A pH screen covering the pH range 4.0 to 7.0 using sodium acetate, histidine acetate, and sodium phosphate as buffers with 240 mM trehalose dihydrate and 0.02% polysorbate 20 showed that Apomab is most stable in solution at pH 6.0. Therefore, a formulation consisting of 20 mM histidine acetate, 240 mM trehalose, 0.02% polysorbate 2, pH 6.0 was developed and demonstrated experimentally to be stable. Using this formulation, Apomab was shown to be stable for at least 12 months at 5°C. Furthermore, Apomab was shown to be stable for at least 12 months at -20°C and three months at 5°C when stored in 316L stainless steel containers. Apomab was also shown to be stable when subjected to up to 3 freeze/thaw cycles.

WHAT IS CLAIMED IS:

1. A stable pharmaceutical formulation comprising a monoclonal antibody in histidine-acetate buffer, pH 5.5 to 6.5.

2. The formulation of claim 1 wherein the pH is from 5.8 to 6.2.

3. The formulation of claim 1 wherein the histidine-acetate buffer concentration is from about 1mM to about 200mM.

4. The formulation of claim 3 wherein the histidine-acetate buffer concentration is from about 10mM to about 40mM.

5. The formulation of claim 1wherein the antibody concentration is from about 10mg/mL to about 250mg/mL.

6. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 20mg/mL to about 40mg/mL.

7. The formulation of claim 5 wherein the monoclonal antibody concentration is from about 80mg/mL to about 250mg/mL.

8. The formulation of claim 1 further comprising saccharide.

9. The formulation of claim 8 wherein the saccharide is a disaccharide.

10. The formulation of claim 8 wherein the saccharide is trehalose.

11. The formulation of claim 8 wherein the saccharide is sucrose.

12. The formulation of claim 8 wherein the saccharide concentration is from about 10mM to about 1M.

13. The formulation of claim 12 wherein the saccharide concentration is from about 60mM to about 250mM.

14. The formulation of claim 1 further comprising surfactant.

15. The formulation of claim 14 wherein the surfactant is polysorbate.

16. The formulation of claim 15 wherein the surfactant is polysorbate 20.

17. The formulation of claim 14 wherein the surfactant concentration is from about 0.0001% to about 1.0%.

18. The formulation of claim 17 wherein the surfactant concentration is from about 0.01% to about 0.1%.

19. The formulation of claim 1 wherein the monoclonal antibody is a full length antibody.

20. The formulation of claim 19 wherein the monoclonal antibody is an IgG1 antibody.

21. The formulation of claim 1 wherein the monoclonal antibody is a humanized antibody.

22. The formulation of claim 1 wherein the monoclonal antibody is an antibody fragment comprising an antigen-binding region.

23. The formulation of claim 22 wherein the antibody fragment is a Fab or F(ab')2 fragment.

24. The formulation of claim 1 which is sterile.

25. The formulation of claim 1 wherein the monoclonal antibody binds an antigen selected from the group consisting of HER2, CD20, DR5, BR3, IgE, and VEGF.

26. The formulation of claim 25 wherein the antigen is CD20 and the monoclonal antibody is humanized 2H7.

27. The formulation of claim 25 wherein the antigen is VEGF and the monoclonal antibody is Bevacizumab.

28. The formulation of claim 1 wherein the monoclonal antibody is susceptible to deamidation or aggregation.

29. The formulation of claim 1 which is stable upon storage at about 40°C for at least 4 weeks

30. The formulation of claim 1 which is stable upon storage at about 5°C or about 15°C for at least 3 months.

31. The formulation of claim 1 which is stable upon storage at about -20°C for at least 3 months.

32. The formulation of claim 1 which is stable upon freezing and thawing.

33. The formulation of claim 1 which is aqueous.

34. The formulation of claim 1 which is frozen.

35. The formulation of claim 1 which is not lyophilized and has not been subjected to prior lyophilization.

36. The formulation of claim 35 which is aqueous and is administered to a subject.

37. The formulation of claim 36 wherein the formulation is for intravenous (IV), subcutaneous (SQ) or intramuscular (IM) administration.

38. The formulation of claim 37 which is for IV administration and the antibody concentration is from about 20mg/mL to about 40mg/mL.

39. The formulation of claim 37 which is for SQ administration and the antibody concentration is from about 80mg/mL to about 250mg/mL.

40. A vial with a stopper pierceable by a syringe comprising the formulation of claim 1 inside the vial.

41. The vial of claim 40 which is stored at about 2-8°C.

42. The vial of claim 40 which is a 20cc or 50cc vial.

43. A stainless steel tank comprising the formulation of claim 1 inside the tank.

44. The tank of claim 43 wherein the formulation therein is frozen.

45. A method of treating a disease or disorder in a subject comprising administering the formulation of claim 1 to a subject in an amount effective to treat the disease or disorder.

46. A pharmaceutical formulation comprising:

(a) a full length IgG1 antibody susceptible to deamidation or aggregation in an amount from about 10mg/mL to about 250mg/mL;

(b) histidine-acetate buffer, pH 5.5 to 6.5;

(c) saccharide selected from the group consisting of trehalose and sucrose, in an amount from about 60mM to about 250mM: and

(d) polysorbate 20 in an amount from about 0.01% to about 0.1%.

47. A method for reducing deamidation or aggregation of a therapeutic monoclonal antibody, comprising formulating the antibody in a histidine-acetate buffer, pH 5.5 to 6.5

48. The method of claim 47 comprising evaluating any antibody deamidation or aggregation before and after the antibody is formulated.

49. A pharmaceutical formulation comprising an antibody that binds to domain II of HER2 in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

50. The formulation of claim 49 wherein the buffer is histidine-acetate.

51. The formulation of claim 49 wherein the HER2 antibody comprises the variable light and variable heavy amino acid sequences in SEQ ID Nos. 3 and 4, respectively.

52. The formulation of claim 51 wherein the HER2 antibody comprises a light chain amino acid sequence selected from SEQ ID No. 15 and 23, and a heavy chain amino acid sequence selected from SEQ ID No. 16 and 24.

53. The formulation of claim 49 wherein the pH of the formulation is from about 5.8 to about 6.2.

54. The formulation of claim 49 wherein the antibody binds to the junction between domains I, II and III of HER2.

55. The formulation of claim 49 wherein the antibody is a full length antibody.

56. The formulation of claim 49 wherein the antibody concentration is from about 20mg/mL to about 40mg/mL.

57. A pharmaceutical formulation comprising Pertuzumab in an amount from about 20mg/mL to about 40mg/mL, histidine-acetate buffer, sucrose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

58. The formulation of claim 57 comprising about 30mg/mL Pertuzumab, about 20mM histidine-acetate, about 120mM sucrose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

59. A vial with a stopper pierceable by a syringe comprising the formulation of claim 49.

60. A stainless steel tank comprising the formulation of claim 49 in the tank.

61. A method of treating HER2-expressing cancer in a subject, comprising administering the pharmaceutical formulation of claim 49 to the subject in an amount effective to treat the cancer.

62. The method of claim 61 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

63. A method of making a pharmaceutical formulation comprising:

(a) preparing the formulation of claim 1; and

(b) evaluating physical stability, chemical stability, or biological activity of the monoclonal antibody in the formulation.

64. A pharmaceutical formulation comprising a DR5 antibody in a histidine buffer at a pH from about 5.5 to about 6.5, a saccharide, and a surfactant.

65. The formulation of claim 64 wherein the buffer is histidine-acetate.

66. The formulation of claim 64 wherein the DR5 antibody is an agonist antibody.

67. The formulation of claim 64 wherein the DR5 antibody is Apomab.

68. The formulation of claim 67 wherein the DR5 antibody comprises the heavy chain amino acid sequence of SEQ ID No. 51, and light chain amino acid sequence of SEQ ID No. 52.

69. The formulation of claim 64 wherein the pH of the formulation is from about 5.8 to about 6.2.

70. The formulation of claim 64 wherein the antibody is a full length antibody.

71. The formulation of claim 64 wherein the antibody concentration is from about 10mg/mL to about 30mg/mL.

72. A pharmaceutical formulation comprising Apomab in an amount from about 10mg/mL to about 30mg/mL, histidine-acetate buffer, trehalose, and polysorbate 20, wherein the pH of the formulation is from about 5.5 to about 6.5.

73. The formulation of claim 72 comprising about 20mg/mL Apomab, about 20mM histidine acetate, about 240mM trehalose, and about 0.02% polysorbate 20, wherein the pH of the formulation is about 6.0.

74. A vial with a stopper pierceable by a syringe comprising the formulation of claim 64.

75. A stainless steel tank comprising the formulation of claim 64 in the tank.

76. A method of treating cancer in a subject, comprising administering the pharmaceutical formulation of claim 64 to the subject in an amount effective to treat the cancer.

77. The method of claim 76 wherein the cancer is a solid tumor.

78. The method of claim 76 wherein the cancer is non-Hodgkin's lymphoma.

79. The method of claim 76 wherein the formulation is administered to the subject intravenously, subcutaneously, or intramuscularly.

ANTIBODY FORMULATIONS

Abstract of the Disclosure

The present application describes antibody formulations, including monoclonal antibodies formulated in histidine-acetate buffer, as well as a formulation comprising an antibody that binds to domain II of HER2 (for example, Pertuzumab), and a formulation comprising an antibody that binds to DR5 (for example, Apomab).

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

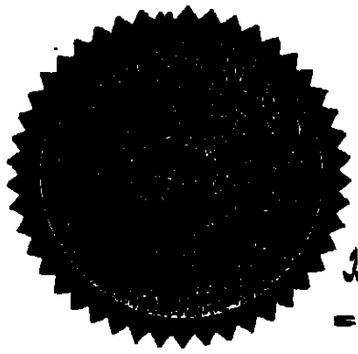
5. at Dover, Delaware

6 the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254030

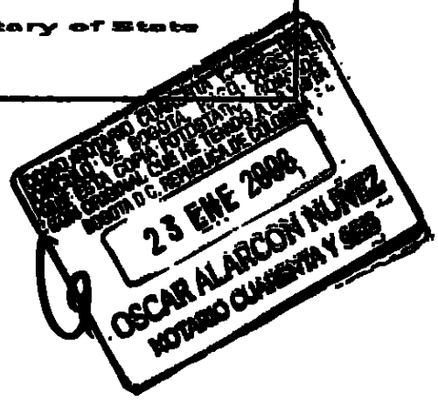
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE RESTATED CERTIFICATE OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE TWENTY-SECOND DAY OF JULY, A.D. 1999, AT 9 O'CLOCK A.M.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

AUTHENTICATION: 3900384

050426506

DATE: 05-24-

23 ENE 2006

OSCAR ALARCON NUÑEZ

515
517

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
991302331

23 ENE 2006

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NOTARIO CUARENTA Y SEIS

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

23 ENE 2008
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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;

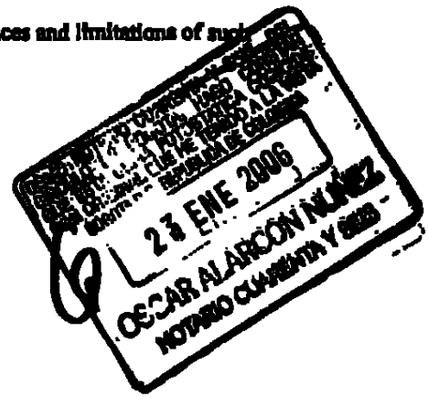
(iv) whether such series shall have conversion privileges and, if so, the terms and conditions of such conversion, including, but not limited to, provision for adjustment of the conversion rate upon such events and in such manner as the Board of Directors shall determine;

(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

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUAUHTEMOC Y MEX.

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

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO GUARENTA Y SEIS

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

23 ENE 2008
OSCAR ALARCÓN NÚÑEZ
NOTARIO CUADRAGÉSIMO Y SEIS

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.

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUADRAGESIMA Y

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

23 ENE 2006
OSCAR ALARCÓN NÚÑEZ
NOTARIO CUARENTA Y OCHO

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 9.05.

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.

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y

526

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.

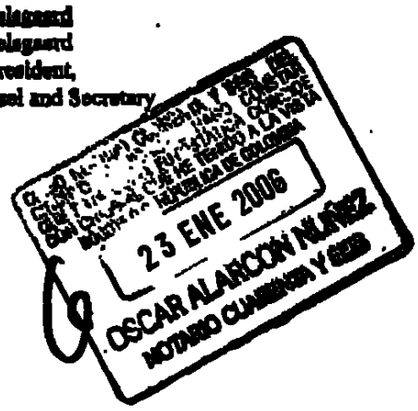


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IN WITNESS WHEREOF, this Amended and Restated Certificate of Incorporation, having been duly adopted in accordance with Sections 242 and 245 of the General Corporation Law of the State of Delaware and by the written consent of the sole stockholder 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



TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc.

APOSTILLA

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

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254030
9. Sello/timbre 10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado



579

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.



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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



preferenciales, por un valor de $0.02 por acción ("Acción Preferencial"). Trecientos millones (300,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("Acción Común"). La acción común de la Corporación será toda de una misma clase.

(b) El número de acciones autorizadas de cualquier clase o clases de acciones puede incrementarse o disminuirse (pero no por debajo del número del número de acciones de las mismas que en su momento están vigentes) por el voto afirmativo de una mayoría con poder de votación de las acciones vigentes de la Corporación con derecho a votar por la misma independiente de las consideraciones de la Sección 242(b)(2) de la Ley de Delaware.

SECCION 4.02. *Acción Común.* (a) *Emisión y Consideración.* Cualquier acción sin emitir o del tesoro del las Acciones Comunes puede emitirse para tal consideración como pueda fijarse de acuerdo con la ley aplicable cada cierto tiempo por la Junta Directiva.

(b) *Dividendos.* Sujeto a los derechos de los accionistas de las Acciones Preferenciales, los accionistas de Acciones Comunes deberán tener el derecho a recibir, cuando y como se declare por la Junta Directiva, de los activos de la Corporación los cuales están disponibles para ello por ley, dividendos pagables ya sea en dinero, propiedad, o acciones y los accionistas de la Acción Preferencial no tendrán derecho a participar en tales dividendos (a menos que la Junta Directiva determine lo contrario en cualquier resolución que condiciona la emisión de una serie de Acciones Preferenciales).

SECCIÓN 4.03. *Acción Preferencial*

(a) *Series y Limites de Variaciones entre Series.* Cualquier acción no emitida o del tesoro de las Acciones Preferenciales puede emitirse cada cierto tiempo en una o más series para tal consideración como pueda ser fijada cada cierto tiempo por la Junta Directiva. Antes de que se emita cualquier acción de las Acciones Preferenciales de cualquier serie particular, se deberá presentar un certificado ante el Secretario de Estado de Delaware estableciendo las designaciones, derechos, privilegios, restricciones, y condiciones que irán a

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y OCHO

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;

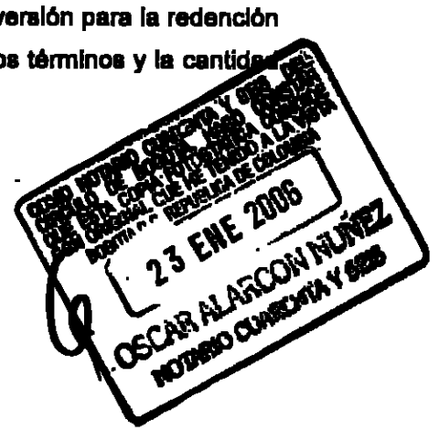
(ii) la tasa anual de dividendos a pagar sobre las acciones de tal serie, las condiciones sobre las cuales se pagarán tales dividendos y la fecha a partir de la cual los dividendos serán acumulativos en el evento que la Junta Directiva determine que los dividendos serán acumulativos;

(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;



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

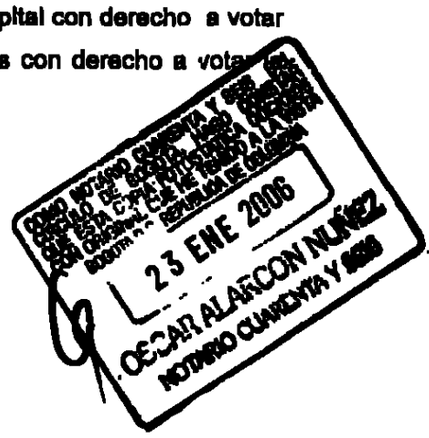
SECCION 5.01. *Enmiendas a los estatutos por los Directores.* (a) En adición y no limitando los poderes conferidos por estatutos, la Junta Directiva esta expresamente autorizada pará hacer, alterar, enmendar, rechazar y rescindir los estatutos de la Corporación, sujetos a las secciones 5.01(b) y 5.01(c) a continuación.

(b) Las secciones 2.02, 3.02, 3.03(a), 3.04, 3.05, 3.14 y 5.06 de los estatutos solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 60% de las acciones con derecho a votar las mismas.

(c) Las secciones 3.03(c) y 3.15 de los estatutos solo pueden ser alteradas, enmendadas, repelidas, o rescindidas por le voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 90% de las acciones con derecho a votar las mismas.

SECCION 5.02. *Enmienda del Certificado de Incorporación.* La sección 5.01 (b) de este Certificado de Incorporación solo puede ser alterada, enmendada, repelida, o rescindida por el voto afirmativo de los accionistas de capital con derecho a voto sobre la misma que representen a más del 60% de las acciones con derecho a votar la misma. La sección 5.01(c) y los Artículos 8 y 9 de este Certificado de Incorporación solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar la misma que representen más del 90% de las acciones con derecho a votar la misma.

ARTICULO 6

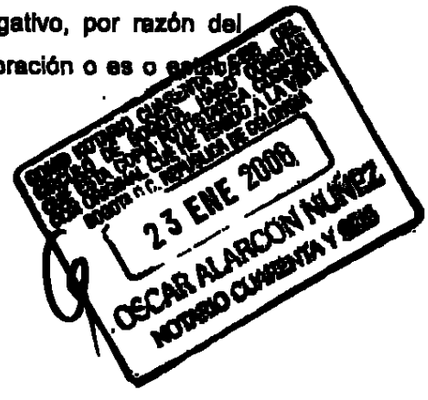


acción preferencial cualquiera de una o más series de las mismas, que voten como una clase, tengan derecho a elegir un número específico de directores, por razón a atrasos en los dividendos u otras condiciones que les dan el derecho a hacerlo, entonces y durante el tiempo que continúe el derecho (I) 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 especifico 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



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 permita 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.



ARTICULO 8

SECCION 8.01. *Relación con Roche.* Anticipándose a que la Corporación cese de ser un subsidiaria completamente de propiedad de Roche Holdings, Inc., pero que Roche (como se define en la sección 8.05) permanezca siendo un accionista de la Corporación, y anticipándose a que la Corporación (como se define en la Sección 8.05) y Roche puede entrar en actividades o líneas de negocios semejantes o similares y tener un interés en las mismas áreas de oportunidades corporativas, y en reconocimiento de (I) los beneficios a ser derivados por la Corporación a través de sus relaciones contractuales, corporativas y de negocios con Roche (incluyendo el servicio de funcionarios y directores de Roche como directores de la Corporación) y (II) las dificultades concomitantes a cualquier director, quien desea y se propone satisfacer completamente tales deberes fiduciarios del director, al determinar el alcance completo de tales deberes en cualquier situación particular, las condiciones de este artículos 8 se establecen para regular, definir y guiar la conducta de ciertas cuestiones de la Corporación ya que pueden involucrar a Roche y sus funcionaros y directores, y los poderes, derechos, deberes y responsabilidades de la Corporación y sus funcionarios, directores y accionistas en conexión con éste.

SECCION 8.02. *Actividades en Negocios Similares.* Excepto que Roche Holdings, Inc. pueda de otra forma acordarlo por escrito,

(a) Roche no tendrá el deber de abstenerse de entrar directamente o indirectamente en actividades de negocios o líneas de negocios semejantes o similares a las de la Corporación, y

(b) ni Roche ni ninguno de sus funcionarios o director de la misma será responsable a la Corporación o sus accionistas por violar cualquier deber fiduciario por razón de alguna de tales actividades de Roche o de la participación de tal persona en las mismas.

En el evento que Roche adquiera conocimientos de una transacción o materia potencial que pueda ser una oportunidad corporativa tanto para Roche como para la Corporación, Roche no tendrá el deber de comunicar u ofrecer



que Roche prosigue o adquiere tal oportunidad corporativa para sí 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 sí 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, Inc Holdings, Inc y todas las corporaciones, sociedades, sociedades de

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUADRAGESIMA Y

define en la Regla 12b-2 bajo el Acta de Intercambio de Valores de 1934, como se enmendó) de Roche Holdings, Inc.

SECCION 8.06, *Finalización: Efecto Obligante.* Son consideración con nada en este Certificado de Incorporación que lo contradiga, las condiciones anteriores de este Artículo 8 deberán expirar en la fecha que Roche cese de poseer con beneficios Acciones Comunes que representen al menos 5% del número de acciones vigentes de las Acciones Comunes de la Corporación y nadie que se director o funcionario de la Corporación sea también director o funcionario de Roche. Ni tal vencimiento, ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 8 así como tampoco ninguna condición de este Certificado de Incorporación Enmendado y Vuelto a Redactar inconsistente con alguna de las condiciones de este Artículo 8 eliminará o reducirá el efecto de este Artículo 8 respecto a cualquier materia, o cualquier causa de acción, demanda o reclamo que, excepto por este Artículo 8, pueda acaecer o surgir, antes de tal vencimiento, alteración, enmienda o adopción.

SECCION 8.07. *Artículo 9.* Las condiciones de este Artículo son adicionales a las condiciones del Artículo 9.

ARTICULO 9

SECCION 9.01. *Contratos no nulos.* Ningún contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización de los mismos) entre la Corporación y Roche será nulo u anulable solamente por razón de que Roche es parte del mismo, o solamente porque cualquiera de los directores o funcionarios de la Corporación quienes están afiliados a Roche están presentes en o participan en la reunión de la Junta Directiva o comité de la misma que autoriza el contrato, acuerdo, arreglo, transacción, enmienda, modificación o finalización o solamente porque su o sus votos se cuentan para tal propósito, pero cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo) estará gobernado por las condiciones de este Cerificado de Incorporación Enmendado y vuelto a Redactar, los Estatutos de la Corporación, la Ley de Delaware y otras leyes aplicables. Para propósitos de este Artículo 9, los términos "***Corporación***" y "***Roche***" tienen los significados establecidos

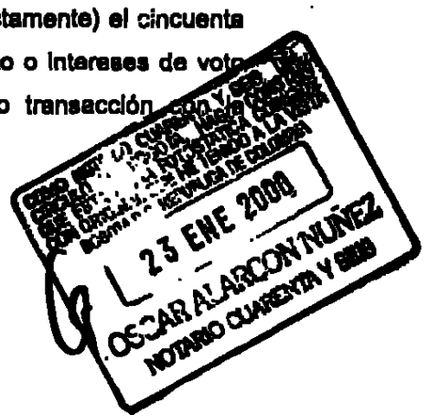


SECCION 9.02. *Quórum.* Los Directores de la Corporación que son también directores o funcionarios de Roche pueden contarse al determinar la presencia de un quórum en una reunión de la Junta Directiva o de un comité que autoriza o aprueba cualquiera de tal contrato, acuerdo, arreglo o transacción (o 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 c 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



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 Secretaria

23 ENE 2006
CESAR ALARCON MUÑEZ
NOTARIO CUARENTA Y OCHO

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

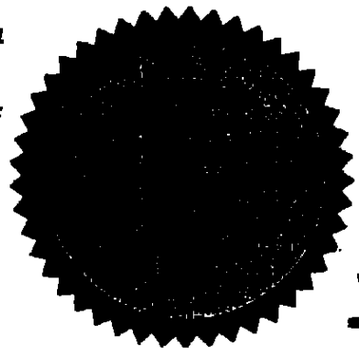
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254031

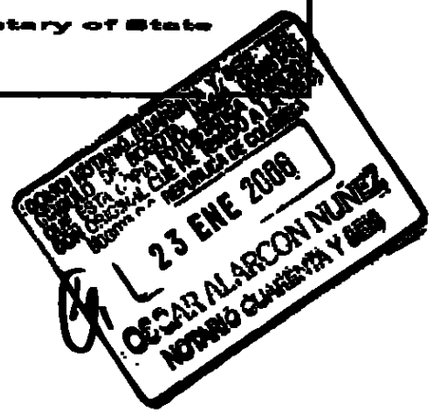
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



542

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE FIFTEENTH DAY OF MAY, A.D. 2000, AT 5 O'CLOCK P.M.

2112549

050426506

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 3900380

DATE: 05-22-00

23 ENE 2006

OSCAR ALARCON NUÑEZ

NOTARIO CUARENTA Y OCHO

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STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 03:00 PM 05/15/2000
001245251 – 2112549

CERTIFICATE OF AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> "Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is seven hundred million (700,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Six hundred million (600,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 15th day of May, 2000.

GENENTECH, INC.

By: Stephen G. Juelsgaard
Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel and Secretary

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23 ENE 2009
OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y SEIS

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TRADUCCION AL ESPAÑOL del Certificado de Incorporación de Genentech, Inc.

APOSTILLA

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

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254031
9. Sello/timbre
10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado



545

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

Harriet Smith Windsor, Secretaria

23 ENE 2006

OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y

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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



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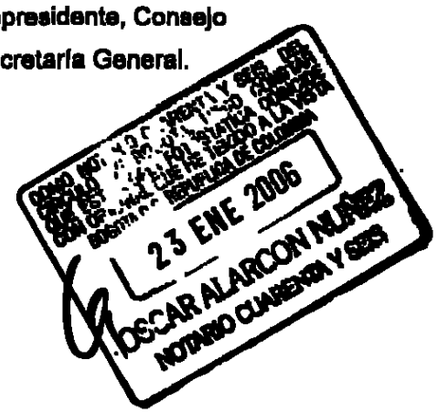
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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.



548

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

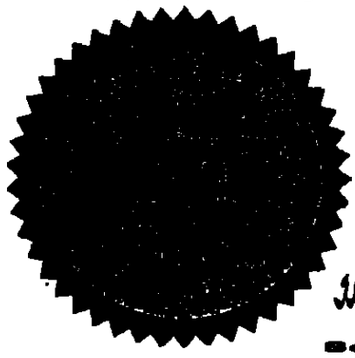
5. at Dover, Delaware

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



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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.

2112549

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Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 3900387

DATE: 05-24-[illegible]

COMO FUNCIONARIO CONSULAR DE LOS ESTADOS UNIDOS DE AMERICA DOY FE QUE EL SELLO Y FIRMA QUE APARECEN EN EL PRESENTE DOCUMENTO SON AUTENTICOS
CONSULADO DE LOS ESTADOS UNIDOS DE AMERICA
23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y [illegible]

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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 - 2112349 FILE

CERTIFICATE OF THIRD AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc., a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware,

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is three billion one hundred million (3,100,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Three billion (3,000,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The common stock of the Corporation shall be all of one class.

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Third Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 16th day of April, 2004.

GENENTECH, INC.

By: Stephen Juelsgaard

Name: Stephen G. Juelsgaard

Title: Executive Vice President, General Counsel and Secretary

#153087

23 ENE 2006

OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y

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TRÁDUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

APOSTILLA

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

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254033
9. Sello/timbre
10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado

23 ENE 2006
OSCAR ALARCÓN NUÑEZ
NOTARIO CUARENTA Y

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

23 ENE 2008

OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y

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CERTIFICADO DE TERCERA ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

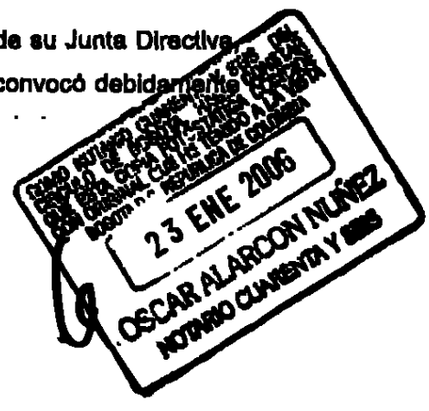
CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de tres mil cien millones (3,100,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Tres mil millones (3,000,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

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



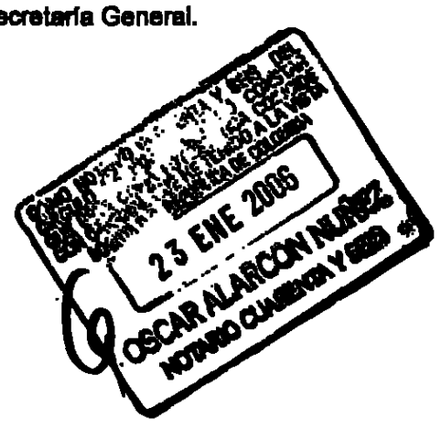
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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.



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(1)

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

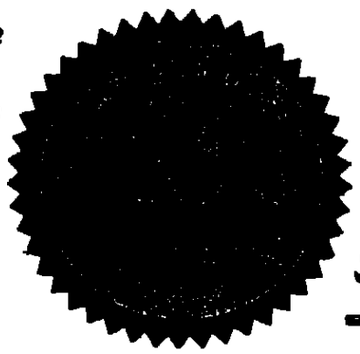
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254032

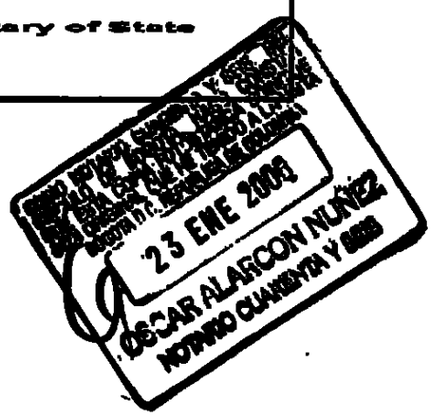
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



554
556

Delaware

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE TENTH DAY OF MAY, A.D. 2001, AT 2:05 O'CLOCK P.M.

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900386

DATE: 05-24-01

OSCAR ALARCON NUÑEZ
NOTARIO CUARENTA Y OCHO
23 ENE 2006

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 03:05 PM 05/10/2001
010225224 – 2112369

S ////STATE/OF/DELAWARE///// V
T ///SECRETARY/OF/STATE/////// O
A DIVISION/OF/CORPORATIONS// I
M FILED/03:05/PM/05/10/2001/ D
P ///RECEIVED//////////
BY Sandy Miller

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

86747

23 ENE 2006
OSCAR ALARCON NUÑEZ
NOTARIO CUADRAGESIMO Y SEIS

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



457
559

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 (Firmado)

Harriet Smith Windsor, Secretaria de Estado

23 ENE 2006

OSCAR ALARCON NUÑEZ

NOTARIO CUARENTA Y SEIS

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.

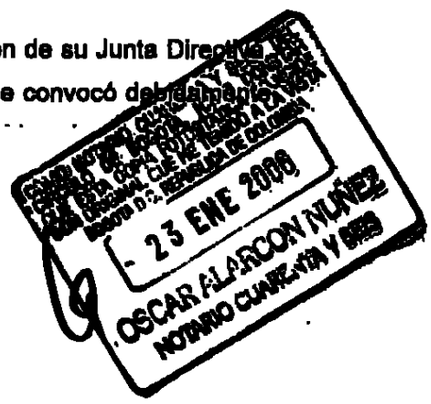
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PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda 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:

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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



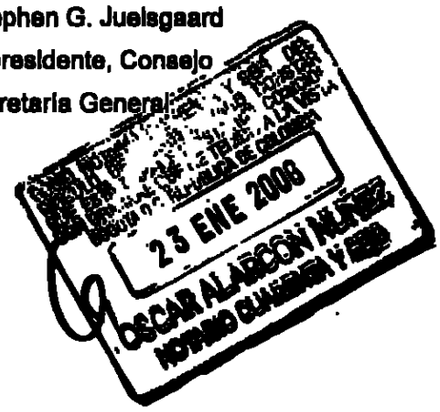
TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

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 Secretaria General



562

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

[redacted]

No. 07-038013- -00000-0000

Fecha: 2007-04-17 16:48:33 Dep: 2020 NUECREACION
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 559

Industria y Comercio
SUPERINTENDENCIA

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 [X]
- 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 [ ]

Claudia M Nova Cortes
Firma Responsable

Fecha_____

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

563

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
CADENA SARMIENTO JUAN PABLO
Apoderado(a) y/o Representante de
GENENTECH, INC.

REFERENCIA	Radicación No.	7 38013
	Solicitud internacional	PCT/US2005/037471
	Fecha inicio fase nacional	20/05/2007
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	5047

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.

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 CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 04 MAYO 2007
adpa11