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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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71. **SOLICITANTE** GENENTECH INC.

DOMICILIO SOUTH SAN FRANCISCO, CA, ESTADOS UNIDOS DE AMERICA

74. **APODERADO** JUAN PABLO CADENA SARMIENTO

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

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1 SOLICITUD DE:

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☒ Capítulo I. ☐ Capítulo II.

2 SOLICITANTE (71)	Nombre: **GENENTECH, INC.** Dirección: 1 DNA Way, South San Francisco, CA 94080-4990, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail	**IDENTIFICACION** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número ____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Carrera 7 # 71 – 21 Torre B – Piso 4 Teléfono: 744-2200 Fax: 744-2700 E-mail:	**IDENTIFICACION** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número 80.410.337 TP 57492
4 INVENTOR (ES) (72)	Nombre: Paul A. Frohna Dirección: 3880 19th Street, San Francisco, CA 94114, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos de América	

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10 **ANEXOS**

- [x] Comprobante de pago de la tasa de presentación de la solicitud. $463.000
- [x] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- [x] Poderes, si fuere el caso. **Fotocopia Autenticada**
- [ ] Documento de cesión del inventor al solicitante o a su causante.
- [ ] Declaraciones
- [x] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [x] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar efectuado por la IPEA.
- [ ] 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: Tarjeta de Propietarios
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11 FIGURA CARACTERÍSTICA:

12 Solicito la concesión de la patente,

NOMBRE: JUAN PABLO CADENA SARMIENTO

FIRMA:

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1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	463,000.00
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(74) Agents: LEE, Wendy, M. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).

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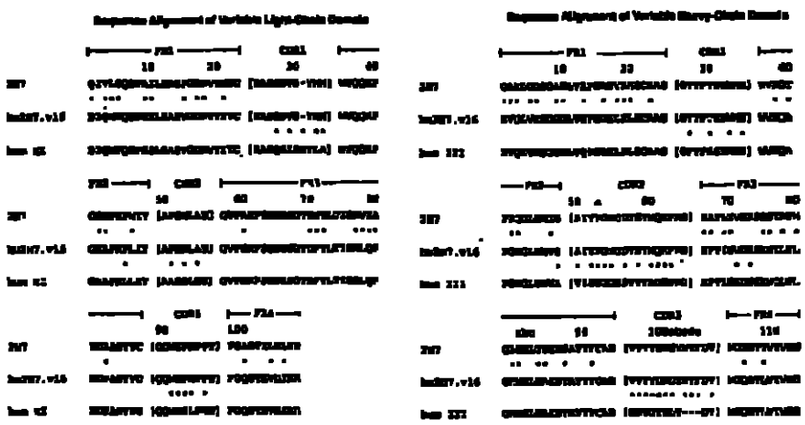
(54) Title: METHOD FOR TREATING MULTIPLE SCLEROSIS



(57) Abstract: Methods for treating multiple sclerosis (MS) with a CD20 antibody using special dosing regimens and protocols are described. Articles of manufacture for use in such methods are also described.

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METHOD FOR TREATING MULTIPLE SCLEROSIS

Field of the Invention

The present invention concerns methods for treating multiple sclerosis (MS) in a subject using special dosing regimens and protocols, and an article of manufacture with instructions for such use.

Background of the Invention

Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory and demyelinating degenerative disease of the human central nervous system (CNS). It is a worldwide disease that affects approximately 300,000 persons in the United States; it is a disease of young adults, with 70%–80% having onset between 20 and 40 years old (Anderson *et al. Ann Neurology* 31(3):333–6 (1992); Noonan *et al. Neurology* 58:136–8 (2002)). MS is a heterogeneous disorder based on clinical course, magnetic resonance imaging (MRI) scan assessment, and pathology analysis of biopsy and autopsy material (Lucchinetti *et al. Ann Neurol* 47:707–17 (2000)). The disease manifests itself in a large number of possible combinations of deficits, including spinal cord, brainstem, cranial nerve, cerebellar, cerebral, and cognitive syndromes. Progressive disability is the fate of most patients with MS, especially when a 25-year perspective is included. Half of MS patients require a cane to walk within 15 years of disease onset. MS is a major cause of neurologic disability in young and middle-aged adults and, until the past decade, has had no known beneficial treatments. MS is difficult to diagnose because of the non-specific clinical findings, which led to the development of highly structured diagnostic criteria that include several technological advances, consisting of MRI scans, evoked potentials, and cerebrospinal fluid (CSF) studies. All diagnostic criteria rely upon the general principles of scattered lesions in the central white matter occurring at different times and not explained by other etiologies such as infection, vascular disorder, or autoimmune disorder (McDonald *et al. Ann Neurol* 50:121–7 (2001)). MS has four patterns of disease: relapsing-remitting MS (RRMS; 80%–85% of cases at onset), primary progressive MS (PPMS; 10%–15% at onset), progressive relapsing MS (PRMS; 5% at onset); and secondary progressive MS (SPMS) (Kremenchutzky *et al. Brain* 122 (Pt 10):1941–50 (1999); Confavreux *et al. N Engl J Med* 343(20):1430–8 (2000)). An estimated 50% of patients with RRMS will develop SPMS in 10 years, and up to 90% of RRMS patients will eventually develop SPMS (Weinshenker *et al. Brain* 112(Pt 1):133–46 (1989)).

Currently, six drugs in four classes are approved in the United States for the treatment of RRMS, whereas no drugs have been approved for PPMS. The RRMS treatments include the following: interferon class, IFN-beta-1a (REBIF® and AVONEX®) and IFN-beta-1b (BETASERON®); glatiramer acetate (COPAXONE®), a polypeptide; natalizumab (TYSABRI®); and mitoxantrone (NOVANTRONE®), a cytotoxic agent. Other drugs have been used with varying

degrees of success, including corticosteroids, methotrexate, cyclophosphamide, azathioprine, and intravenous (IV) immunoglobulin. The benefits of currently approved treatments are relatively modest (~30%) for relapse rate and prevention of disability in RRMS as suggested by two recent meta-analyses (Filippini *et al. Lancet* 361:545–52 (2003)).

Other clinical studies evaluated other immunomodulatory agents in MS, including tumor necrosis factor–α inhibitors and altered peptide ligands, which aggravated rather than improved MS (Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI *Neurology* 53:457–65 (1999); Bielekova *et al. Nat Med* 2000;6:1167–75 (2000), erratum appears in *Nat Med* 6:1412 (2000)).

The predominant view of MS pathophysiology has held that inflammation is principally mediated by $CD4^+$ Th1 T cells. Therapeutic approaches based on this theory such as IFN-beta and glatiramer acetate decrease, but do not fully prevent, occurrence of exacerbations or accumulation of disability.

The existence of a humoral component in human MS has been implicitly recognized for decades, as evidenced by inclusion of CSF oligoclonal bands and increased intrathecal IgG synthesis in diagnostic criteria for MS (Siden A. *J Neurol* 221:39–51(1979); McDonald *et al. Ann Neurol* 50:121–7 (2001); Andersson *et al. Eur J Neurol* 9:243–51 (2002); O'Connor, P. *Neurology* 59:S1–33 (2002)). The presence of oligoclonal bands, increased free light chains, and increased intrathecal IgM synthesis correlates with MS disease activity and may be a predictor of more severe outcomes (Rudick *et al. Mult Scler* 1:150–5 (1995); Zeman *et al. Acta Cytol* 45:51–9 (2001); Izquierdo *et al. Acta Neurol Scand* 105:158–63 (2002); Wolinsky J. *J Neurol Sci* 206:145–52 (2003); Villar *et al. Ann Neurol* 53:222–6 (2003)).

Anti-myelin antibodies (myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG)) have been detected in the serum of patients with progressive and relapsing forms of MS (Reindl *et al. Brain* 122:2047–56 (1999); Egg *et al. Mult Scler* 7(5):285–9 (2001)). Anti-myelin antibodies have also been detected in the CSF of MS patients (Reindl *et al. Brain* 122:2047–56 (1999); Egg *et al. Mult Scler* 7(5):285–9 (2001); Andersson *et al. Eur J Neurol* 9:243–51 (2002)). Additional types of antibodies such as anti-ganglioside antibodies or anti-neurofilament antibodies have been observed in patients with MS (Mata *et al. Mult Scler* 5:379–88 (1999); Sadatipour *et al. Ann Neurol* 44:980–3 (1998)). A recent report indicated that the presence of serum anti-MOG and anti-MBP antibodies was a strong predictor of progression from a clinically isolated demyelinating event to definite RRMS (Berger *et al. N Engl J Med* 349:139–45 (2003)). The adjusted hazard ratio for experiencing an exacerbation was 76.5 for patients who were seropositive for both antibodies and 31.6 for patients who were seropositive only for anti-MOG.

An international pathology consortium found that antibodies bound to myelin are present in the majority of patients with MS, with plasma cells and B cells also found in MS lesions, providing

additional evidence for a humoral role in MS (Prineas and Wright, *Lab Invest* 38:409–21 (1978); Esiri M. *Neuropathol Appl Neurobiol* 6:9–21 (1980); Genain *et al. Nat Med* 5:170–5 (1999); Lucchinetti *et al. Ann Neurol* 47:707–17 (2000); Wingerchuk *et al. Lab Invest* 81:263–81 (2001)). B cells are detectable in the CSF of patients with MS, and the presence of a relatively high proportion of B cells may be predictive of more severe disability progression (Cepok *et al. Brain* 124(Pt 11):2169–76 (2001)).

In subjects with RRMS or opsoclonus-myoclonus syndrome, Rituximab reportedly depleted peripheral B cells in all subjects and decreased the number of CSF B cells in some patients (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003); Cross *et al.* "Preliminary Results from a Phase II Trial of Rituximab in MS" (abstract) *Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis ACTRIMS* 20–1 (October, 2003)). See also Cree *et al.* "Tolerability and Effects of Rituximab "Anti-CD20 Antibody" in Neuromyelitis Optica and Rapidly Worsening Multiple Sclerosis" *Meeting of the Am. Acad. Neurol.* (April, 2004).

CD20 Antibodies and Therapy Therewith

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

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

The CD20 antigen (also called human B-lymphocyte-restricted differentiation antigen, Bp35) is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes (Valentine *et al. J. Biol. Chem.* 264(19):11282-11287 (1989); and Einfeld *et al. EMBO J.* 7(3):711-717 (1988)). The antigen is also expressed on greater than 90% of B-cell non-Hodgkin's lymphomas (NHL) (Anderson *et al. Blood* 63(6):1424-1433 (1984)), but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues (Tedder *et al. J. Immunol.* 135(2):973-979 (1985)). CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation (Tedder *et al., supra*) and possibly functions as a calcium ion channel (Tedder *et al. J. Cell. Biochem.* 14D:195 (1990)).

Given the expression of CD20 in B-cell lymphomas, this antigen can serve as a candidate for "targeting" of such lymphomas. In essence, such targeting can be generalized as follows: antibodies specific to the CD20 surface antigen of B cells are administered to a patient. These anti-CD20

antibodies specifically bind to the CD20 antigen of (ostensibly) both normal and malignant B cells; the antibody bound to the CD20 surface antigen may lead to the destruction and depletion of neoplastic B cells. Additionally, chemical agents or radioactive labels having the potential to destroy the tumor can be conjugated to the anti-CD20 antibody such that the agent is specifically "delivered" to the neoplastic B cells. Irrespective of the approach, a primary goal is to destroy the tumor; the specific approach can be determined by the particular anti-CD20 antibody that is utilized and, thus, the available approaches to targeting the CD20 antigen can vary considerably.

The Rituximab (RITUXAN®) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen. Rituximab is the antibody called "C2B8" in US Patent No. 5,736,137 issued April 7, 1998 (Anderson *et al.*). RITUXAN® is indicated for the treatment of patients with relapsed or refractory low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma. *In vitro* mechanism of action studies have demonstrated that RITUXAN® binds human complement and lyses lymphoid B-cell lines through complement-dependent cytotoxicity (CDC) (Reff *et al. Blood* 83(2):435-445 (1994)). Additionally, it has significant activity in assays for antibody-dependent cellular cytotoxicity (ADCC). More recently, RITUXAN® has been shown to have anti-proliferative effects in tritiated thymidine incorporation assays and to induce apoptosis directly, while other anti-CD19 and CD20 antibodies do not (Maloney *et al. Blood* 88(10):637a (1996)). Synergy between RITUXAN® and chemotherapies and toxins has also been observed experimentally. In particular, RITUXAN® sensitizes drug-resistant human B-cell lymphoma cell lines to the cytotoxic effects of doxorubicin, CDDP, VP-16, diphtheria toxin and ricin (Demidem *et al. Cancer Chemotherapy & Radiopharmaceuticals* 12(3):177-186 (1997)). *In vivo* preclinical studies have shown that RITUXAN® depletes B cells from the peripheral blood, lymph nodes, and bone marrow of cynomolgus monkeys, presumably through complement and cell-mediated processes (Reff *et al. Blood* 83(2):435-445 (1994)).

Rituximab was approved in the United States in November 1997 for the treatment of patients with relapsed or refractory low-grade or follicular $CD20^+$ B-cell non-Hodgkin's lymphoma (NHL) at a dose of 375 mg/m^2 weekly for four doses. In April 2001, the Food and Drug Administration (FDA) approved additional claims for the treatment of low-grade NHL: retreatment (weekly for four doses) and an additional dosing regimen (weekly for eight doses). There have been more than 300,000 patient exposures to Rituximab either as monotherapy or in combination with immunosuppressant or chemotherapeutic drugs. Patients have also been treated with Rituximab as maintenance therapy for up to 2 years (Hainsworth *et al. J Clin Oncol* 21:1746–51 (2003); Hainsworth *et al. J Clin Oncol* 20:4261–7 (2002)).

Rituximab has also been studied in a variety of non-malignant autoimmune disorders, in which B cells and autoantibodies appear to play a role in disease pathophysiology (Edwards *et al. Biochem Soc Trans* 30:824–8 (2002)). Rituximab has been reported to potentially relieve signs and symptoms of rheumatoid arthritis (RA) (Leandro *et al. Ann Rheum Dis.* 61:883–8 (2002); Emery *et*

al. Arthritis Rheum 48(9):S439 (2003)), lupus (Eisenberg R. *Arthritis Res Ther* 5:157–9 (2003); Leandro *et al. Arthritis Rheum* 46:2673–7 (2002)), immune thrombocytopenia (D'Arena *et al. Leuk Lymphoma* 44:561–2 (2003)), autoimmune anemia (Zaja *et al. Haematologica* 87:189–95 (2002) (erratum appears in *Haematologica* 87:336 (2002)), autoimmune neuropathy (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485–9 (2003)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), and relapsing-remitting multiple sclerosis (RRMS) (Cross *et al.* (abstract) Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis 20–1 (2003)).

A Phase II study (WA16291) has been conducted in patients with rheumatoid arthritis (RA), providing 48-week follow-up data on safety and efficacy of Rituximab (Emery *et al. Arthritis Rheum* 48(9):S439 (2003); Szczepanski *et al. Arthritis Rheum* 48(9):S121 (2003)). A total of 161 patients were evenly randomized to four treatment arms: methotrexate, Rituximab alone, Rituximab plus methotrexate, Rituximab plus cyclophosphamide (CTX). The treatment regimen of Rituximab was 1 g administered intravenously on Days 1 and 15. Infusions of Rituximab in most patients with RA were well tolerated by most patients, with 36% of patients experiencing at least one adverse event during their first infusion (compared with 30% of patients receiving placebo). Overall, the majority of adverse events were considered to be mild to moderate in severity and were well balanced across all treatment groups. There were a total of 19 serious adverse events across the four arms over the 48 weeks, which were slightly more frequent in the Rituximab/CTX group. The incidence of infections was well balanced across all groups. The mean rate of serious infection in this RA patient population was 4.6 6 per 100 patient-years, which is lower than the rate of infections requiring hospital admission in RA patients (9.57 per 100 patient-years) reported in a community-based epidemiologic study (Doran *et al. Arthritis Rheum* 46:2287–93 (2002)).

The reported safety profile of Rituximab in a small number of patients with neurologic disorders, including autoimmune neuropathy (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485–9 (2003)), opsoclonus/myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), and RRMS (Cross *et al.* Preliminary results from a phase II trial of Rituximab in MS (abstract) Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis 20–1 (2003)), was similar to that reported in oncology or RA. In an ongoing investigator-sponsored trial (IST) of Rituximab in combination with interferon-beta (IFN-beta) or glatiramer acetate in subjects with RRMS (Cross *et al., supra*), 1 of 10 treated subjects was admitted to the hospital for overnight observation after experiencing moderate fever and rigors following the first infusion of Rituximab, while the other 9 subjects completed the four-infusion regimen without any reported adverse events.

Patents and patent publications concerning CD20 antibodies include US Patent Nos. 5,776,456, 5,736,137, 5,843,439, 6,399,061, and 6,682,734, as well as US 2002/0197255, US 2003/0021781, US 2003/0082172, US 2003/0095963, US 2003/0147885 (Anderson *et al.)*; US Patent

No. 6,455,043, US 2003/0026804, and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); WO 2000/27433 and US 2004/0213784 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO01/10461 (Rastetter and White); WO 2001/10460 (White and Grillo-Lopez); US 2001/0018041, US 2003/0180292, WO 2001/34194 (Hanna and Hariharan); US 2002/0006404 and WO 2002/04021 (Hanna and Hariharan); US 2002/0012665 and WO 2001/74388 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858 (White, C.); US 2002/0128488 and WO 2002/34790 (Reff, M.); WO 2002/060955 (Braslawsky *et al.*);WO 2002/096948 (Braslawsky *et al.*);WO 2002/079255 (Reff and Davies); US Patent No. 6,171,586 and WO 1998/56418 (Lam *et al.*); WO 1998/58964 (Raju, S.); WO 1999/22764 (Raju, S.); WO 1999/51642, US Patent No. 6,194,551, US Patent No. 6,242,195, US Patent No. 6,528,624 and US Patent No. 6,538,124 (Idusogie *et al.*); WO 2000/42072 (Presta, L.); WO 2000/67796 (Curd *et al.*); WO 2001/03734 (Grillo-Lopez *et al.*); US 2002/0004587 and WO 2001/77342 (Miller and Presta); US 2002/0197256 (Grewal, I.); US 2003/0157108 (Presta, L.); WO 04/056312 (Lowman *et al.*); US 2004/0202658 and WO 2004/091657 (Benyunes, K.); WO 2005/000351 (Chan, A.); US 2005/0032130A1 (Beresini *et al.*); US 2005/0053602A1 (Brunetta, P.); US Patent Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721, (Kaminski *et al.*); US Patent Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, and 6,652,852 (Robinson *et al.*); US Pat No. 6,410,391 (Raubitschek *et al.*); US Patent No. 6,224,866 and WO00/20864 (Barbera-Guillem, E.); WO 2001/13945 (Barbera-Guillem, E.); US2005/0079174A1 (Barbera-Guillem *et al.*); WO 2000/67795 (Goldenberg); US 2003/0133930 and WO 2000/74718 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO2004/058298 (Goldenberg and Hansen); WO 2000/76542 (Golay *et al.*);WO 2001/72333 (Wolin and Rosenblatt); US Patent No. 6,368,596 (Ghetie *et al.*); US Patent No. 6,306,393 and US 2002/0041847 (Goldenberg, D.); US 2003/0026801 (Weiner and Hartmann); WO 2002/102312 (Engleman, E.); US 2003/0068664 (Albitar *et al.*); WO 2003/002607 (Leung, S.); WO 2003/049694, US2002/0009427, and US 2003/0185796 (Wolin *et.al.*); WO 2003/061694 (Sing and Siegall); US 2003/0219818 (Bohen *et al.*); US 2003/0219433 and WO 2003/068821 (Hansen *et al.*); US 2003/0219818 (Bohen *et al.*); US2002/0136719 (Shenoy *et al.*); WO 2004/032828 (Wahl *et al.*); and WO 2002/56910 (Hayden-Ledbetter). See also US Patent No. 5,849,898 and EP 330,191 (Seed *et al.*); EP332,865A2 (Meyer and Weiss); US Patent No. 4,861,579 (Meyer *et al.*); US2001/0056066 (Bugelski *et al.*); WO 1995/03770 (Bhat *et al.*); US 2003/0219433 A1 (Hansen *et al.*); WO 2004/035607 (Teeling *et al.*); US 2004/0093621 (Shitara *et al.*); WO 2004/103404 (Watkins *et al.*); WO 2005/000901 (Tedder *et al.*); US 2005/0025764 (Watkins *et al.*); WO2005/016969 and US 2005/0069545 A1 (Carr *et al.*); WO 2005/014618 (Chang *et al.*). Certain of these include, *inter alia*, treatment of multiple sclerosis.

Publications concerning therapy with Rituximab include: Perotta and Abuel "Response of chronic relapsing ITP of 10 years duration to Rituximab" Abstract # 3360 *Blood* 10(1)(part 1-2): p.

88B (1998); Stashi *et al.* "Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idopathic thrombocytopenic purpura" *Blood* 98(4):952-957 (2001); Matthews, R. "Medical Heretics" *New Scientist* (7 April, 2001); Leandro *et al.* "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" *Ann Rheum Dis* 61:833-888 (2002); Leandro *et al.* "Lymphocyte depletion in rheumatoid arthritis: early evidence for safety, efficacy and dose response. *Arthritis and Rheumatism* 44(9): S370 (2001); Leandro *et al.* "An open study of B lymphocyte depletion in systemic lupus erythematosus", *Arthritis & Rheumatism* 46(1):2673-2677 (2002); Edwards and Cambridge "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rheumatology* 40:205-211 (2001); Edwards *et al.* "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem. Soc. Trans.* 30(4):824-828 (2002); Edwards *et al.* "Efficacy and safety of Rituximab, a B-cell targeted chimeric monoclonal antibody: A randomized, placebo controlled trial in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 46(9): S197 (2002); Levine and Pestronk "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab" *Neurology* 52: 1701-1704 (1999); DeVita *et al.* "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hidashida *et al.* "Treatment of DMARD-Refractory rheumatoid arthritis with Rituximab." Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Tuscano, J. "Successful treatment of Infliximab-refractory rheumatoid arthritis with Rituximab" Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001); Anolik *et al.*, "B lympocyte Depletion in the Treatment of Systemic Lupus (SLE): Phase I/II Trial of Rituximab (RITUXAN®) in SLE" *Arthritis And Rheumatism*, 46(9), S289-S289 Abstract 717 (October, 2002), and Albert *et al.*, "A Phase I Trial of Rituximab (Anti-CD20) for Treatment of Systemic Lupus Erythematosus" *Arthritis And Rheumatism*, 48(12): 3659-3659, Abstract LB9 (December, 2003); Martin and Chan "Pathogenic Roles of B cells in Human Autoimmunity: Insights from the Clinic" *Immunity* 20:517-527 (2004).

Summary of the Invention

The present invention involves, at least in part, the selection of a dose for a CD20 antibody that provides a safe and active treatment regimen in subjects with multiple sclerosis, such as PPMS or RRMS.

Accordingly, the invention concerns a method of treating multiple sclerosis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial

exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

In addition, the invention concerns an article of manufacture comprising: (a) container comprising a CD20 antibody; and (b) a package insert with instructions for treating multiple sclerosis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being administered until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

Brief Description of the Drawings

FIG. 1A is a sequence alignment comparing the amino acid sequences of the light chain variable domain (V_L) of each of murine 2H7 (SEQ ID NO:1), humanized 2H7.v16 variant (SEQ ID NO:2), and the human kappa light chain subgroup I (SEQ ID NO:3). The CDRs of V_L of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:5), and CDR3 (SEQ ID NO:6).

FIG. 1B is a sequence alignment comparing the amino acid sequences of the heavy chain variable domain (V_H) of each of murine 2H7 (SEQ ID NO:7), humanized 2H7.v16 variant (SEQ ID NO:8), and the human consensus sequence of the heavy chain subgroup III (SEQ ID NO:9). The CDRs of V_H of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12).

In FIG. 1A and FIG. 1B, the CDR1, CDR2 and CDR3 in each chain are enclosed within brackets, flanked by the framework regions, FR1-FR4, as indicated. 2H7 refers to the 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.

FIG. 2 shows the amino acid sequence of the mature 2H7.v16 light chain (SEQ ID NO:13)

FIG. 3 shows the amino acid sequence of the mature 2H7.v16 heavy chain (SEQ ID NO:14).

FIG. 4 shows the amino acid sequence of the mature 2H7.v31 heavy chain (SEQ ID NO:15). The L chain of 2H7.v31 is the same as for 2H7.v16.

FIG. 5 shows an alignment of the mature 2H7.v16 and 2H7.v511 light chains (SEQ ID NOS. 13 and 16, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

FIG. 6 shows an alignment of the mature 2H7.v16 and 2H7.v511 heavy chains (SEQ ID NOS. 14 and 17, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Detailed Description of the Preferred Embodiments

I. Definitions

A "B-cell" is a lymphocyte that matures within the bone marrow, and includes a naive B cell, memory B cell, or effector B cell (plasma cells). The B-cell herein may be a normal or non-malignant B cell.

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.

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.

An "antibody antagonist" herein is a antibody that, upon binding to a B cell surface marker on B cells, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antibody antagonist preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC), inhibition of B-cell proliferation and/or induction of B-cell death (*e.g.* via apoptosis).

"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 that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes that 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 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 that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term 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" refer 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.

"Growth inhibitory" antibodies are those that prevent or reduce proliferation of a cell expressing an antigen to which the antibody binds. For example, the antibody may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

Antibodies that "induce apoptosis" are those that induce programmed cell death, *e.g.* of a B cell, as determined by standard apoptosis assays, such as 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 term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding 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 fragments.

For the purposes herein, an "intact antibody" is one comprising heavy and light variable domains as well as an Fc region.

"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).

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 that 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 that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

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

"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 that 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).

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

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 (immunoglobulins) 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; 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, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

"Humanized" forms of non-human (*e.g.*, murine) 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, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, 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).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region 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 Leak *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

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

Examples of antibodies that bind the CD20 antigen include: "C2B8," which is now called "Rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from IDEC Pharmaceuticals, Inc. (US Patent No. 5,736,137; 2B8 deposited with ATCC under accession no. HB11388 on June 22, 1993); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "^{131}I-B1" or "iodine I131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, US Patent No. 5,595,721); 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 (US Patent No. 5,677,180); humanized 2H7; huMax-CD20 (Genmab, Denmark, WO2004/035607); AME-133 (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) or IMMU-106 (US 2003/0219433, Immunomedics); CD20-binding antibodies, including epitope-depleted Leu-16, 1H4, or 2B8, optionally conjugated with IL-2, as in US 2005/0069545A1 and WO 2005/16969 (Carr *et al.*); bispecific antibody that binds CD22 and CD20, for example, hLL2xhA20 (WO2005/14618, Chang *et al.*); 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)); 1H4 (Haisma *et al. Blood* 92:184 (1998)); anti-CD20 auristatin E conjugate (Seattle Genetics); anti-CD20-IL2 (EMD/Biovation/City of Hope); anti-CD20 MAb therapy (EpiCyte); and anti-CD20 antibody TRU 015 (Trubion).

The terms "Rituximab" or "RITUXAN®" herein refer to the genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen and designated "C2B8" in US Patent No. 5,736,137, including fragments thereof that retain the ability to bind CD20. Rituximab is commercially available from Genentech.

Purely for the purposes herein and unless indicated otherwise, "humanized 2H7" refers to a humanized antibody that binds human CD20, or an antigen-binding fragment thereof, wherein the antibody is effective to deplete primate B cells *in vivo*, the antibody comprising in the H chain variable region (V_H) thereof at least a CDR H3 sequence of SEQ ID NO:12 (Fig. 1B) from an anti-human CD20 antibody and substantially the human consensus framework (FR) residues of the human heavy- chain subgroup III (V_HIII). In a preferred embodiment, this antibody further comprises the H chain CDR H1 sequence of SEQ ID NO:10 and CDR H2 sequence of SEQ ID NO:11, and more preferably further comprises the L chain CDR L1 sequence of SEQ ID NO:4, CDR L2 sequence of SEQ ID NO:5, CDR L3 sequence of SEQ ID NO:6 and substantially the human consensus framework (FR) residues of the human light chain subgroup I (VI), wherein the V_H region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3. In a preferred embodiment, such antibody comprises the V_H sequence of SEQ ID NO:8 (v16, as shown in Fig. 1B), optionally also comprising the V_L sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), which may have the amino acid substitutions of D56A and N100A in the H chain and S92A in the L chain (v96). Preferably the antibody is an intact antibody comprising the light and heavy chain amino acid sequences of SEQ ID NOS:13 and 14, respectively, as shown in Figs. 2 and 3. Another preferred embodiment is where the antibody is 2H7.v31 comprising the light and heavy chain amino acid sequences of SEQ ID NOS:13 and 15, respectively, as shown in Figs. 2 and 4. The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC and/or CDC activity, such as one wherein the amino acid substitutions are S298A/E333A/K334A, more preferably 2H7.v31 having the heavy chain amino acid sequence of SEQ ID NO:15 (as shown in Fig. 4). Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that decreases CDC activity, for example, comprising at least the substitution K322A. See U.S. Patent No. 6,528,624B1 (Idusogie *et al.)*.

A preferred humanized 2H7 is an intact antibody or antibody fragment comprising the variable light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and the variable heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY

NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SS (SEQ ID NO:8).

Where the humanized 2H7 antibody is an intact antibody, preferably it comprises the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

and the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

In the preferred embodiment of the invention, the variable region of variants based on 2H7 version 16 will have 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.

2H7 Version	Heavy chain (V_H) changes	Light chain (V_L) changes	Fc changes
31	-	-	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N10	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous 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 "subject" herein is a human subject. Generally, the subject is eligible for treatment for multiple sclerosis. For the purposes herein, such eligible subject is one who is experiencing, has experienced, or is likely to experience, one or more signs, symptoms or other indicators of multiple sclerosis; has been diagnosed with multiple sclerosis, whether, for example, newly diagnosed (with "new onset" MS), previously diagnosed with a new relapse or exacerbation, previously diagnosed and in remission, etc; and/or is at risk for developing multiple sclerosis. One suffering from or at risk for suffering from multiple sclerosis may optionally be identified as one who has been screened for elevated levels of CD20-positive B cells in serum, cerebrospinal fluid (CSF) and/or MS lesion(s) and/or is screened for using an assay to detect autoantibodies, assessed qualitatively, and preferably quantitatively. Exemplary such autoantibodies associated with multiple sclerosis include anti-myelin basic protein (MBP), anti-myelin oligodendrocytic glycoprotein (MOG), anti-ganglioside and/or anti-neurofilament antibodies. Such autoantibodies may be detected in the subject's serum, cerebrospinal fluid (CSF) and/or MS lesion. By "elevated" autoantibody or B cell level(s) herein is meant level(s) of such autoantibodies or B cells which significantly exceed the level(s) in an individual without MS.

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

A "symptom" of MS is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the subject and indicative of MS.

"Multiple sclerosis" refers to the chronic and often disabling disease of the central nervous system characterized by the progressive destruction of the myelin. There are four internationally

recognized forms of MS, namely, primary progressive multiple sclerosis (PPMS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS).

"Primary progressive multiple sclerosis" or "PPMS" is characterized by a gradual progression of the disease from its onset with no superimposed relapses and remissions at all. There may be periods of a leveling off of disease activity and there may be good and bad days or weeks. PPMS differs from RRMS and SPMS in that onset is typically in the late thirties or early forties, men are as likely women to develop it, and initial disease activity is often in the spinal cord and not in the brain. PPMS often migrates into the brain, but is less likely to damage brain areas than RRMS or SPMS; for example, people with PPMS are less likely to develop cognitive problems. PPMS is the sub-type of MS that is least likely to show inflammatory (gadolinium enhancing) lesions on MRI scans. The Primary Progressive form of the disease affects between 10 and 15% of all people with multiple sclerosis. PPMS may be defined according to the criteria in McDonald *et al. Ann Neurol* 50:121–7 (2001). The subject with PPMS treated herein is usually one with probable or definitive diagnosis of PPMS.

"Relapsing-remitting multiple sclerosis" or "RRMS" is characterized by relapses (also known as exacerbations) during which time new symptoms can appear and old ones resurface or worsen. The relapses are followed by periods of remission, during which time the person fully or partially recovers from the deficits acquired during the relapse. Relapses can last for days, weeks or months and recovery can be slow and gradual or almost instantaneous. The vast majority of people presenting with MS are first diagnosed with RRMS. This is typically when they are in their twenties or thirties, though diagnoses much earlier or later are known. Twice as many women as men present with this sub-type of MS. During relapses, myelin, a protective insulating sheath around the nerve fibers (neurons) in the white matter regions of the central nervous system (CNS), may be damaged in an inflammatory response by the body's own immune system. This causes a wide variety of neurological symptoms that vary considerably depending on which areas of the CNS are damaged. Immediately after a relapse, the inflammatory response dies down and a special type of glial cell in the CNS (called an oligodendrocyte) sponsors remyelination - a process whereby the myelin sheath around the axon may be repaired. It is this remyelination that may be responsible for the remission. Approximately 50% of patients with RRMS convert to SPMS within 10 years of disease onset. After 30 years, this figure rises to 90%. At any one time, the relapsing-remitting form of the disease accounts around 55% of all people with MS.

"Secondary progressive multiple sclerosis" or "SPMS" is characterized by a steady progression of clinical neurological damage with or without superimposed relapses and minor remissions and plateaux. People who develop SPMS will have previously experienced a period of RRMS which may have lasted anything from two to forty years or more. Any superimposed relapses and remissions there are, tend to tail off over time. From the onset of the secondary progressive phase

of the disease, disability starts advancing much quicker than it did during RRMS though the progress can still be quite slow in some individuals. After 10 years, 50% of people with RRMS will have developed SPMS. By 25 to 30 years, that figure will have risen to 90%. SPMS tends to be associated with lower levels of inflammatory lesion formation than in RRMS but the total burden of disease continues to progress. At any one time, SPMS accounts around 30% of all people with multiple sclerosis.

"Progressive relapsing multiple sclerosis" refers to "PRMS" is characterized by a steady progression of clinical neurological damage with superimposed relapses and remissions. There is significant recovery immediately following a relapse but between relapses there is a gradual worsening of symptoms. PRMS affects around 5% of all people with multiple sclerosis. Some neurologists believe PRMS is a variant of PPMS.

The expression "effective amount" refers to an amount of the antibody (or other drug) that is effective for preventing, ameliorating or treating the multiple sclerosis. Such an effective amount will generally result in an improvement in the signs, symptoms or other indicators of MS, such as reducing relapse rate, preventing disability, reducing number and/or volume of brain MRI lesions, improving timed 25-foot walk, extending the time to disease progression (*e.g.* using Expanded Disability Status Scale, EDSS), etc.

"Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses administered over a period of time of about 1-20 days. The doses may be given at one time or at fixed or irregular time intervals over this period of exposure. Initial and later (*e.g.* second or third) antibody exposures are separated in time from each other as described in detail herein.

The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, down-regulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (see U.S. Pat. No. 4,665,077); nonsteroidal anti-inflammatory drugs (NSAIDs); ganciclovir, tacrolimus, glucocorticoids such as cortisol or aldosterone, anti-inflammatory agents such as a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist; purine antagonists such as azathioprine or mycophenolate mofetil (MMF); alkylating agents such as cyclophosphamide; bromocryptine; danazol; dapsone; glutaraldehyde (which masks the MHC antigens, as described in U.S. Pat. No. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); hydroxycloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antagonists including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor-alpha antibodies (infliximab or adalimumab), anti-TNF-alpha immunoadhesin (etanercept), anti-tumor necrosis factor-beta antibodies, anti-interleukin-2

antibodies and anti-IL-2 receptor antibodies; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 90/08187 published 7/26/90); streptokinase; TGF-beta; streptodornase; RNA or DNA from the host; FK506; RS-61443; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, U.S. Pat. No. 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294; Ianeway, *Nature*, 341: 482 (1989); and WO 91/01133); and T cell receptor antibodies (EP 340,109) such as T10B9.

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, or fragments 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 CYTOXAN® cyclosphosphamide; 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); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; 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, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as

methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abberant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2;

LURTOTECAN® topoisomerase I inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; 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-11, IL-12, IL-15; 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, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "hormone" refers to polypeptide hormones, which are generally secreted by glandular organs with ducts. Included among the hormones are, for example, 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); prolactin, placental lactogen, mouse gonadotropin-associated peptide, inhibin; activin; mullerian-inhibiting substance; and thrombopoietin. As used herein, the term hormone includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence hormone, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "growth factor" refers to proteins that promote growth, and include, for example, hepatic growth factor; fibroblast growth factor; vascular endothelial growth factor; nerve growth factors such as NGF-β; platelet-derived 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; and colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF). As used herein, the term growth factor includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence growth factor, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells and apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits, called alpha and beta, that are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include Alpha6beta1,

Alpha3beta1, Alpha4beta1, LFA-1, alpha 4 integrin etc. As used herein, the term integrin includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence integrin, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody such as Efalizumab (RAPTIVA®) commercially available from Genentech; an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen; diazacyclic phenylalanine derivatives (WO 2003/89410); phenylalanine derivatives (WO 2003/70709, WO 2002/28830, WO 2002/16329 and WO 2003/53926); phenylpropionic acid derivatives (WO 2003/10135); enamine derivatives (WO 2001/79173); propanoic acid derivatives (WO 2000/37444); alkanoic acid derivatives (WO 2000/32575); substituted phenyl derivatives (US Pat. Nos. 6,677,339 and 6,348,463); aromatic amine derivatives (US Pat. No. 6,369,229); and ADAM disintegrin domain polypeptide (US2002/0042368), antibodies to alphavbeta3 integrin (EP 633945); aza-bridged bicyclic amino acid derivatives (WO 2002/02556) etc.

For the purposes herein, "tumor necrosis factor alpha (TNF-alpha)" refers to a human TNF-alpha molecule comprising the amino acid sequence as described in Pennica *et al.*, *Nature*, 312:721 (1984) or Aggarwal *et al.*, *JBC*, 260:2345 (1985).

A "TNF-alpha inhibitor" herein is an agent that inhibits, to some extent, a biological function of TNF-alpha, generally through binding to TNF-alpha and neutralizing its activity. Examples of TNF inhibitors specifically contemplated herein are Etanercept (ENBREL®), Infliximab (REMICADE®) and Adalimumab (HUMIRA™).

Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methotrexate), azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, Staphylococcal protein A immunoadsorption, including salts and derivatives thereof, etc.

Examples of "nonsteroidal anti-inflammatory drugs" or "NSAIDs" are acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, including salts and derivatives thereof, etc.

"Corticosteroid" refers to any one of several synthetic or naturally occurring substances with the general chemical structure of steroids that mimic or augment the effects of the naturally occurring corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone), dexamethasone, glucocorticoid and betamethasone.

A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage,

administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products, etc.

II. Therapy

The present invention provides a method of treating multiple sclerosis in a subject suffering therefrom, comprising administering an effective amount of an antibody that binds to a B-cell surface marker (preferably a CD20 antibody) to the subject. The MS to be treated herein includes primary progressive multiple sclerosis (PPMS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS), but therapy of either PPMS or RRMS are the preferred embodiments herein.

According to a preferred dosing protocol, the method comprises administering an effective amount of a CD20 antibody to the MS subject to provide an initial antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams) followed by a second antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams), the second antibody exposure not being provided until from about 16 to 60 weeks from the initial antibody exposure. For purposes of this invention, the second antibody exposure is the next time the subject is treated with the CD20 antibody after the initial antibody exposure, there being no intervening CD20 antibody treatment or exposure between the initial and second exposures.

The interval between the initial and second or subsequent antibody exposures can be measured from either the first or second dose of the initial antibody exposure, but preferably from the first dose of the initial antibody exposure.

In the preferred embodiments herein, the antibody exposures are approximately 24 weeks or 6 months apart; or approximately 48 weeks or 12 months apart.

In one embodiment, the second antibody exposure is not provided until about 20 to 30 weeks from the initial exposure, optionally followed by a third antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams), the third exposure not being administered until from about 46 to 60 weeks (preferably from about 46 to 54 weeks) from the initial exposure, and then, preferably no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.

In an alternative embodiment, the second antibody exposure is not provided until about 46 to 60 weeks from the initial exposure, and subsequent antibody exposures, if any, are not provided until about 46 to 60 weeks from the previous antibody exposure.

Any one or more of the antibody exposures herein may be provided to the subject as a single dose of antibody, or as two separate doses of the antibody (*i.e.*, constituting a first and second dose). The particular number of doses (whether one or two) employed for each antibody exposure is dependent, for example, on the type of MS treated, the type of antibody employed, whether and what type of second medicament is employed, and the method and frequency of administration. Where two separate doses are administered, the second dose is preferably administered from about 3 to 17 days, more preferably from about 6 to 16 days, and most preferably from about 13 to 16 days from the

time the first dose was administered. Where two separate doses are administered, the first and second dose of the antibody is preferably about 0.5 to 1.5 grams, more preferably about 0.75 to 1.3 grams.

In one embodiment, the subject is provided at least about three, or at least four exposures of the antibody, for example, from about 3 to 60 exposures, and more particularly about 3 to 40 exposures, most particularly, about 3 to 20 exposures. Preferably, such exposures are administered at intervals each of approximately 24 weeks or 6 months, or 48 weeks or 12 months. In one embodiment, each antibody exposure is provided as a single dose of the antibody. In an alternative embodiment, each antibody exposure is provided as two separate doses of the antibody. However, not every antibody exposure need be provided as a single dose or as two separate doses.

The antibody may be a naked antibody or may be conjugated with another molecule such as a cytotoxic agent such as a radioactive compound. The preferred antibody herein is Rituximab, humanized 2H7 (*e.g.* comprising the variable domain sequences in SEQ ID NOS. 2 and 8) or humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 23 and 24, or huMax-CD20 (Genmab).

In one embodiment, the subject has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the multiple sclerosis and/or has never been previously treated with an antibody to a B-cell surface marker (*e.g.* never previously treated with a CD20 antibody).

The antibody is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, *e.g.*, US Patent Appln No. 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antibody. Preferably, the dosing is given intravenously, subcutaneously or intrathecally, most preferably by intravenous infusion(s).

While the CD20 antibody may be the only drug administered to the subject to treat the multiple sclerosis, one may optionally administer a second medicament, such as a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody (*e.g.* an LFA-1 antibody such as efalizumab (RAPTIVA®) commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen) etc, with the antibody that binds a B cell surface marker (*e.g.* with the CD20 antibody).

In the preferred embodiment of combination therapy, the antibody is combined with an interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, azathioprine;

intravenous immunoglobulin (gamma globulin); lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, cladribine, total body irradiation, bone marrow transplantation); corticosteroid (*e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid), including systemic corticosteroid therapy; non–lymphocyte-depleting immunosuppressive therapy (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class, which includes cerivastatin (BAYCOL®), fluvastatin (LESCOL®), atorvastatin (LIPITOR®), lovastatin (MEVACOR®), pravastatin (PRAVACHOL®), Simvastatin (ZOCOR®); estradiol; testosterone (optionally at elevated dosages; Stuve *et al.* *Neurology* 8:290–301 (2002)); hormone replacement therapy; treatment for symptoms secondary or related to MS *(e.g.,* spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); plasmapheresis; levothyroxine; cyclosporin A; somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; rehabilitative surgery; radioiodine; thyroidectomy; another B-cell surface antagonist/antibody; etc.

The second medicament is administered with the initial exposure and/or later exposures of the CD20 antibody, such combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

Aside from administration of antibodies to the subject the present application contemplates administration of antibodies by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression administering an "effective amount" of an antibody. See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the subject's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the subject, usually at the site where the antibody is required. For *ex vivo* treatment, the subject's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the subject either directly or, for example, encapsulated within porous membranes that are implanted into the subject (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro include* the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins that bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

III. Production of Antibodies

The methods and articles of manufacture of the present invention use, or incorporate, an antibody that binds to a B-cell surface marker, especially one that binds to CD20. Accordingly, methods for generating such antibodies will be described here.

The B cell surface marker to be used for production of, or screening for, antibodies may be, *e.g.*, a soluble form of the marker or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing the marker at their cell surface can be used to generate, or screen for, antibodies. Other forms of the B cell surface marker useful for generating antibodies will be apparent to those skilled in the art.

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention.

(i) Polyclonal antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3

volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(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 arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal 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); 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 immunoglobulin 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 immunoglobulin 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, 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- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; 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 that 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 that 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 chain variable regions. 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 that 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.

(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 US 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, US Patent Nos. 5,565,332 and 5,573,905.

Human antibodies may also be generated by *in vitro* activated B cells (see US Patents 5,567,610 and 5,229,275).

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. 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; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US 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 B cell surface marker. Other such antibodies may bind the B cell surface marker and further bind a second different B-cell surface marker. Alternatively, an anti-B cell surface marker binding arm may be combined with an arm that 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 B cell. Bispecific antibodies may also be used to localize cytotoxic agents to the B cell. These antibodies possess a B cell surface marker-binding arm and an arm that 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).

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 US Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that 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 (US 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 US Patent No. 4,676,980, along with a number of cross-linking techniques.

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

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 that 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).

IV. Conjugates and Other Modifications of the Antibody'

The antibody used in the methods or included in the articles of manufacture herein is optionally conjugated to a cytotoxic agent. For instance, the antibody may be conjugated to a drug as described in WO2004/032828.

Chemotherapeutic agents useful in the generation of such antibody-cytotoxic agent conjugates have been described above.

Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (US Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one 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 conjugate.

Alternatively, the antibody is 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 that 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)).

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

The present invention further contemplates antibody conjugated with 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 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 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 subject, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) that is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

The antibodies of the present invention may also be conjugated with a prodrug-activating enzyme that converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of such conjugates includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the antibody by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

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 (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody fragments, such as Fab', linked to one or more PEG molecules are an especially preferred embodiment of the invention.

The antibodies disclosed herein may also be formulated as liposomes. 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 an 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).

Amino acid sequence modification(s) of the antibody 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 an 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 of an enzyme, or a polypeptide that 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 different residue. The sites of greatest interest for substitutional mutagenesis of antibody antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a 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. 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 is 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 in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to 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. Such altering includes 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 polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The 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-acetylgalactosamine, 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) concerning a CD20 antibody composition. 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.

The preferred glycosylation variant herein comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US Pat. Appl. No. US 2003/0157108 A1, Presta, L; WO 00/61739A1; WO01/29246A1; US2003/0115614A1; US2002/0164328A1; US2004/0093621A1; US2004/0132140A1; US2004/0110704A1; US2004/0110282A1; US2004/0109865A1; WO03/085119A1; WO03/084570A1; WO2005/035778; WO2005/035586 (describing RNA inhibition (RNAi) of fucosylation); Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004)).

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

It may be desirable to 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 an antibody 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 that 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.*).

V. Pharmaceutical Formulations

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

sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Exemplary anti-CD20 antibody formulations are described in WO98/56418. This publication describes a liquid multidose formulation comprising 40 mg/mL Rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. Another anti-CD20 formulation of interest comprises 10mg/mL Rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5.

Lyophilized formulations adapted for subcutaneous administration are described in US Pat No. 6,267,958 (Andya *et al.*). Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

Crystalized forms of the antibody or antibody are also contemplated. See, for example, US 2002/0136719A1 (Shenoy *et al.*).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent; chemotherapeutic agent; immunosuppressive agent; cytokine; cytokine antagonist or antibody; growth factor; hormone; integrin; integrin antagonist or antibody (*e.g.* an LFA-1 antibody such as efalizumab/RAPTIVA commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab/TYSABRI®) available from Biogen); interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; testosterone; hormone replacement therapy; drug that treats symptoms secondary or related to MS (*e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid (*e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A; somatastatin analogue; cytokine antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody (*e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4 integrin antibody such as natalizumab); or another B-cell surface antagonist/antibody; etc in the formulation. The type and effective amounts of such other agents depend, for example, on the amount of antibody present in the formulation, the type of multiple sclerosis being treated, and clinical parameters of the subjects. These are generally used in

the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

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

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

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

VI. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of multiple sclerosis described above is provided. Preferably, the article of manufacture comprises:(a) a container comprising a composition comprising an antibody that binds to a B-cell surface marker (*e.g.* a CD20 antibody) and a pharmaceutically acceptable carrier or diluent within the container; and (b) a package insert with instructions for administering the composition to a subject suffering from multiple sclerosis to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial exposure; or instructions for administering the composition to a subject suffering from PPMS.

The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for treating the multiple sclerosis and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the antibody. The label or package insert indicates that the composition is used for treating multiple sclerosis in a subject suffering therefrom with specific guidance regarding dosing amounts and

intervals of antibody and any other drug being provided. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Optionally, the article of manufacture herein further comprises a container comprising an agent other than the antibody for treatment and further comprising instructions on treating the mammal with such agent, such agent preferably being a chemotherapeutic agent or immunosuppressive agent, interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; hormone replacement therapy; drug that treats symptoms secondary or related to MS (*e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid (*e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A; somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody (*e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4 integrin antibody such as natalizumab); and another B-cell surface marker antibody; etc.

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1

TREATMENT OF PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS (PPMS)

A subject with diagnosis of PPMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) is treated with a CD20 antibody in this example.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 24 (Day 169), Week 48 (Day 337), and Week 72 (Day 505). The second infusion of the subsequent courses of treatment will be 14 ± 1 days after the first infusion.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse is defined as all of the following:

- An acute appearance of a neurologic abnormality that persists for at least 24 hours
- A change not attributable to fever, infection, trauma, concomitant medications, or other etiology
- An event with objective change on examination by the blinded examining investigator, including a minimum of 1-point change on one of the following FS scales: pyramidal, cranial nerves, cerebellar, sensory, vision, or gait

The following regimen of corticosteroids may be used: 1 g IV methylprednisolone daily for 3 days, followed by 60 mg prednisone daily for 5 days, and decreasing by 10-mg increments each day thereafter. If IV methylprednisolone is not available, then 150 mg IV dexamethasone daily for 3 days may be substituted. Only one course of corticosteroids should be administered for an exacerbation. Subsequent immunological studies and MRI scans should be obtained at least 4 weeks after completion of the corticosteroid regimen. Corticosteroid inhalers (oral and nasal) or intra-articular injections may be used.

Additional treatments to be optionally combined with the CD20 antibody include IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

The primary efficacy outcome measure is the time to confirmed disease progression. Disease progression is defined as an increase of ≥ 1.0 point from baseline Expanded Disability Status Scale (EDSS) (Kurtzke J. *Neurology* 33(11):1444–52 (1983)), if the baseline EDSS is between 2.0 and 5.5 points (inclusive), or an increase of ≥ 0.5 point if the baseline EDSS is ≥ 5.5 points (inclusive), for which change is not attributable to another etiology (*e.g.*, fever, concurrent illness, MS relapse or exacerbation, or concomitant medication). Confirmation of disease progression may occur at a regularly scheduled visit that is at least 12 weeks (84 days) after the initial progression.

Secondary efficacy outcome measures include:

- Change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan
- Change from baseline to Week 96 in brain volume on brain MRI scan

Optionally, improvement in any one or more of:

- Multiple Sclerosis Functional Composite Scale (MSFCS)
- EDSS
- Proportion of subjects with confirmed disease progression at Week 96, as determined using EDSS
- Upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS)
- Ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS)
- Cognition, as measured by the Paced Auditory Serial Addition Test (3 seconds only; a subscale of the MSFCS)
- Total volume of brain T2 lesions on MRI scans (Weeks 48 and 122)
- Total volume of brain T1 lesions on MRI scans
- Cross sectional area of the cervical spinal cord on MRI scans
- Brain volume on MRI scans (Weeks 48 and 122)

The subject treated with Rituximab as described herein will show improvement in the signs, symptoms or other indicators of PPMS according to any one or more of the above outcome measures.

EXAMPLE 2

TREATMENT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS

Subjects with RRMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) are treated with a CD20 antibody in this example, where the antibody exposures are approximately 6 months apart.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 24 (Day 169), Week 48 (Day 337), and Week 72 (Day 505). The second infusion of the subsequent courses of treatment will be 14 ± 1 days after the first infusion.

Preferably Rituximab is the only agent administered to treat the RRMS. However, subjects may optionally receive IV or oral corticosteroids, IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse in this Example is defined as:

The appearance of new or recurrent neurological symptoms consistent with MS lasting more than 48 hours in a subject who has been in a relatively stable or improving neurologic state for at least 30 days. The change in neurologic symptoms must be accompanied by objective neurologic worsening consistent with an increase of at least half a step on the EDSS, or 2 points on one of the appropriate functional system scores (FSS), or 1 point on two or more of the appropriate FSS. The change must be verified by the examining investigator and must affect the selected FS scales (*i.e.*, pyramidal, gait, cerebellar, brainstem, sensory, or visual). Symptoms must persist for≥ 24 hours and should not be attributable to confounding clinical factors (*e.g.*, fever, infection, injury, adverse reactions to concomitant medications). A single episode of a paroxysmal symptom (*e.g.*, tonic spasm) is not a relapse, but the new onset of multiple occurrences of a paroxysmal symptom over at least 24 hours can be a relapse if accompanied by new, corresponding objective manifestations. Sensory symptoms with no change on clinical examination, fatigue, mood change, or bladder or bowel urgency or incontinence will not be sufficient to establish a relapse.

The primary efficacy outcome measure is the MRI endpoint of gadolinium-enhancing lesions, or time to confirmed disease progression (defined as an increase of ≥ 1.0 point from baseline Expanded Disability Status Scale (EDSS); Kurtzke J. *Neurology* 33(11):1444–52 (1983)). The primary efficacy endpoint may be the total number of gadolinium-enhancing T1 lesions observed on serial MRI scans of the brain at Weeks 12, 16, 20, and 24.

Secondary efficacy outcome measures include frequency of relapse; change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan (*e.g.* change in total volume of T2 lesions on MRA scans of the brain from screening to weeks 24 and 36); change from baseline to Week 96 in brain volume on brain MRI scan; Multiple Sclerosis Functional Composite Scale (MSFCS) and its subscales; upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS); ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS); cognition, as measured by the Paced Auditory Serial Addition Test (a subscale of the MSFCS); Multiple Sclerosis Quality of Life–54 (MSQOL-54) questionnaire; total volume of brain T1 lesions on MRI scans (*e.g.* total number of gadolinium-enhancing T1 lesions observed on serial MRA scans of the brains at weeks 20, 28, and 36); cross sectional area of the cervical spinal cord on MRI scans; proportion of subjects relapsing by weeks 24 (*i.e* between week 0 and week 24) and 36 (*i.e.* between week 0 and week 36); the Combined Unique Activity Measure at week 24 and 36.

The patient treated with Rituximab as described above will display an improvement in any one or more of the above outcome measures.

EXAMPLE 3

TREATMENT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS

A subject with RRMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) is treated with a CD20 antibody herein. In this example, the antibody exposures are approximately 1 year apart.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 48, and Week 96. The second infusion of the subsequent courses of treatment will be 14 ± 1 days after the first infusion.

Preferably Rituximab is the only agent administered to treat the RRMS. However, subjects may optionally receive IV or oral corticosteroids, IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse in this Example is defined as:

The appearance of new or recurrent neurological symptoms consistent with MS lasting more than 48 hours in a subject who has been in a relatively stable or improving neurologic state for at least 30 days. The change in neurologic symptoms must be accompanied by objective neurologic worsening consistent with an increase of at least half a step on the EDSS, or 2 points on one of the appropriate functional system scores (FSS), or 1 point on two or more of the appropriate FSS. The change must be verified by the examining investigator and must affect the selected FS scales (*i.e.*, pyramidal, gait, cerebellar, brainstem, sensory, or visual). Symptoms must persist for≥ 24 hours and should not be attributable to confounding clinical factors (*e.g.*, fever, infection, injury, adverse reactions to concomitant medications). A single episode of a paroxysmal symptom (*e.g.*, tonic spasm) is not a relapse, but the new onset of multiple occurrences of a paroxysmal symptom over at least 24 hours can be a relapse if accompanied by new, corresponding objective manifestations. Sensory symptoms with no change on clinical examination, fatigue, mood change, or bladder or bowel urgency or incontinence will not be sufficient to establish a relapse.

The primary efficacy outcome measure is the MRI endpoint of gadolinium-enhancing lesions, or time to confirmed disease progression (defined as an increase of ≥ 1.0 point from baseline · Expanded Disability Status Scale (EDSS); Kurtzke J. *Neurology* 33(11):1444–52 (1983)). The primary efficacy endpoint may be the total number of gadolinium-enhancing T1 lesions observed on serial MRI scans of the brain at Weeks 12, 16, 20, and 24.

Secondary efficacy outcome measures include frequency of relapse; change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan (*e.g.* change in total volume of T2 lesions on MRA scans of the brain from screening to weeks 24 and 36); change from baseline to Week 96 in brain volume on brain MRI scan; Multiple Sclerosis Functional Composite Scale (MSFCS) and its subscales; upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS); ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS); cognition, as measured by the Paced Auditory Serial Addition Test (a subscale of the MSFCS); Multiple Sclerosis Quality of Life–54 (MSQOL-54) questionnaire; total volume of brain T1 lesions on MRI scans (*e.g.* total number of gadolinium-enhancing T1 lesions observed on serial MRA scans of the brains at weeks 20, 28, and 36); cross sectional area of the cervical spinal cord on MRI scans; proportion of subjects relapsing by week 24 (*i.e* between week 0 and week 24) and week 36 (*i.e.* between week 0 and week 36); the Combined Unique Activity Measure at week 24 and 36.

The patient treated with the above with Rituximab will display an improvement in any one or more of the above outcome measures.

EXAMPLE 4

HUMANIZED 2H7 VARIANTS

This example describes humanized 2H7 antibody variants for use in the methods disclosed herein. The humanized 2H7 antibody preferably 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. 18), for example SEQ ID NO:4 (Fig. 1A),

CDR L2 sequence of SEQ ID NO:5 (Fig. 1A),

CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID NO. 19), for example SEQ ID NO:6 (Fig. 1A),

CDR H1 sequence of SEQ ID NO:10 (Fig. 1B),

CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID NO. 20), for example SEQ ID NO:11 (Fig. 1B), and

CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W or D, and the X as position 7 is S or R (SEQ ID NO. 21), for example SEQ ID NO:12 (Fig. 1B).

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:8 (v16, as shown in Fig. 1B), optionally also comprising the variable light domain sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), 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. 13 or 16, and heavy chain amino acid sequences of SEQ ID NO. 14, 15, 17, 22 or 25.

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:2 and the variable heavy domain of SEQ ID NO:8, 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:8 and a variable light domain with alteration M32L; or S92A; or M32L and S92A; in SEQ ID NO:2.

M34 in the variable heavy domain of 2H7.v16 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 2H7.v16 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 2H7.v16 variable light domain sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and 2H7.v16 variable heavy domain sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV
SS (SEQ ID NO:8).

Where the humanized 2H7.v16 antibody is an intact antibody, it may comprise the light chain amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

and the heavy chain amino acid sequence of SEQ ID NO. 14 or:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG (SEQ ID NO:22).

Another preferred humanized 2H7 antibody comprises 2H7.v511 variable light domain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:23)
and 2H7.v511 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGN
GATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQ
GTLVTVSS (SEQ ID NO. 24).

Where the humanized 2H7.v511 antibody is an intact antibody, it may comprise the light chain amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:16)

and the heavy chain amino acid sequence of SEQ ID NO. 17 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR
VVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 25).

WHAT IS CLAIMED IS:

1. A method of treating multiple sclerosis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

2. The method of claim 1 wherein the second exposure is not provided until from about 20 to 30 weeks from the initial exposure.

3. The method of claim 1 or 2 wherein the initial and second antibody exposures are each provided in amounts of about 1.5 to 2.5 grams.

4. The method of any one of claims 2-3 additionally comprising administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure of about 0.5 to 4 grams, the third exposure not being administered until from about 46 to 60 weeks from the initial exposure.

5. The method of claim 4 wherein the third antibody exposure is provided in an amount of about 1.5 to 2.5 grams.

6. The method of claim 4 or 5 wherein the third exposure is not provided until from about 46 to 54 weeks from the initial exposure.

7. The method of any one of claims 4-6 wherein no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.

8. The method of claim 1 wherein the second exposure is not provided until from about 46 to 60 weeks from the initial exposure.

9. The method of any one of claims 1-8 wherein each of the antibody exposures is provided to the subject as one dose of antibody.

10. The method of any one of claim 1-8 wherein each of the antibody exposures is provided to the subject as two doses of antibody, wherein the two doses constitute a first dose and a second dose.

11. The method of claim 10 wherein the second dose is administered from about 3-17 days from the time the first dose was administered.

12. The method of claim 11 wherein the second dose is administered from about 6-16 days from the time the first dose was administered.

13. The method of claim 12 wherein the second dose is administered from about 13-16 days from the time the first dose was administered.

14. The method of any one of claims 10-13 wherein the first and second dose of antibody are each about 0.5 to 1.5 grams.

15. The method of any one of claims 10-14 wherein the first and second dose of antibody are each about 0.75 to 1.3 grams.

16. The method of any one of the claims 1-15 wherein three or more antibody exposures are administered to the subject.

17. The method of any one of claims 1-16 wherein four or more antibody exposures are administered to the subject

18. The method of any one of claims 1-17 wherein a second medicament is administered with the initial exposure or later exposures, wherein the CD20 antibody is a first medicament.

19. The method of claim 18 wherein the second medicament is selected from the group consisting of an interferon, glatiramer acetate, a cytotoxic agent, chemotherapeutic agent, mitoxantrone, methotrexate, cyclophosphamide, chlorambucil, azathioprine, gamma globulin, Campath, anti-CD4, cladribine, corticosteroid, mycophenolate mofetil (MMF), cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone replacement drug, a TNF inhibitor, disease-modifying anti-rheumatic drug (DMARD), non-steroidal anti-inflammatory drug (NSAID), levothyroxine, cyclosporin A, somatastatin analogue, cytokine or cytokine receptor antagonist, anti-metabolite, immunosuppressive agent, integrin antagonist or antibody, LFA-1 antibody, efalizumab, alpha 4 integrin antibody, natalizumab, and another B-cell surface marker antibody.

20. The method of any one of claims 1-19 wherein the multiple sclerosis is relapsing-remitting multiple sclerosis (RRMS).

21. The method of any one of claims 1-19 wherein the multiple sclerosis is primary progressive multiple sclerosis (PPMS).

22. The method of any one of claims 1-21 wherein the subject has never been previously treated with a CD20 antibody.

23. The method of any one of claims 1-22 wherein the antibody is a naked antibody.

24. The method of any one of claims 1-22 wherein the antibody is conjugated with another molecule.

25. The method of claim 24 wherein the other molecule is a cytotoxic agent.

26. The method of any one of claims 1-25 wherein the antibody is administered intravenously.

27. The method of claim 26 wherein the antibody is administered intravenously for each antibody exposure.

28. The method of any one of claims 1-25 wherein the antibody is administered subcutaneously.

29. The method of any one of claims 1-25 wherein the antibody is administered intrathecally.

30. The method of any one of claims 1-29 wherein the CD20 antibody is the only medicament administered to the subject to treat the multiple sclerosis.

31. The method of any one of claims 1-30 wherein the antibody is Rituximab.

32. The method of any one of claims 1-30 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 2 and 8.

33. The method of any one of claims 1-30 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 23 and 24.

34. The method of any one of claims 1-34 wherein the subject has elevated anti-myelin basic protein (MBP), anti-myelin oligodendrocyte glycoprotein (MOG), anti-ganglioside and/or or anti-neurofilament antibody level(s).

35. The method of any one of claims 1-34 wherein elevated levels of B cells are present in cerebrospinal fluid (CSF), multiple sclerosis lesion, or serum of the subject.

36. An article of manufacture comprising:

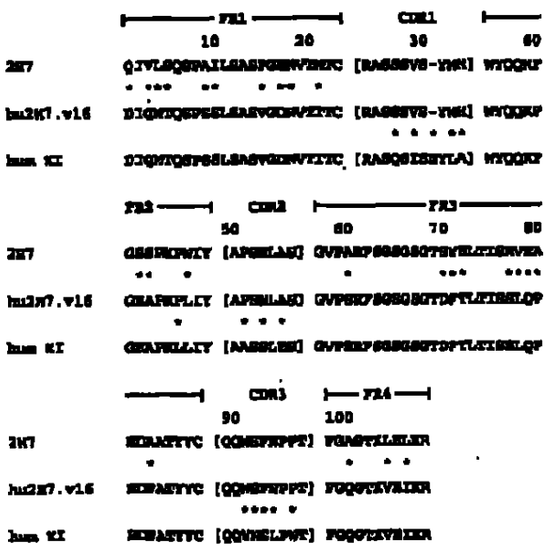
(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating multiple sclerosis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being administered until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

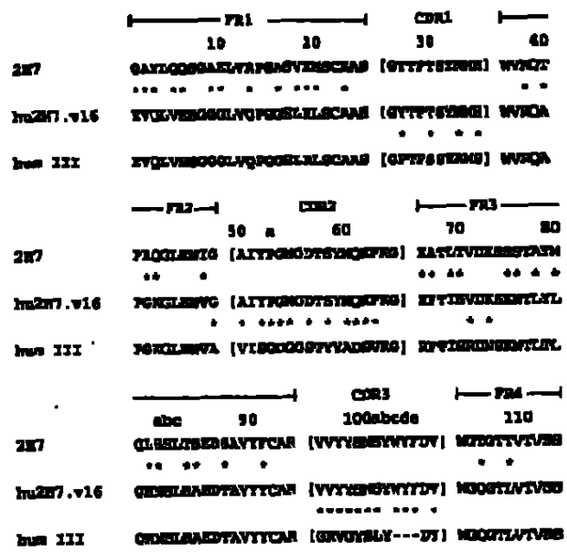
37. The article of claim 36 further comprising a container comprising a second medicament, wherein the CD20 antibody is a first medicament, and further comprising instructions on the package insert for treating the subject with the second medicament.

38. The article of claim 37 wherein the second medicament is selected from the group consisting of an interferon, glatiramer acetate, a cytotoxic agent, chemotherapeutic agent, mitoxantrone, methotrexate, cyclophosphamide, chlorambucil, azathioprine, gamma globulin, Campath, anti-CD4, cladribine, corticosteroid, mycophenolate mofetil (MMF), cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone replacement drug, a TNF inhibitor, disease-modifying anti-rheumatic drug (DMARD), non-steroidal anti-inflammatory drug (NSAID), levothyroxine, cyclosporin A, somatastatin analogue, cytokine or cytokine receptor antagonist, anti-metabolite, immunosuppressive agent, and another B-cell surface marker antibody.

Sequence Alignment of Variable Light-Chain Domain



Sequence Alignment of Variable Heavy-Chain Domain



Sequence Alignment of Variable Heavy-Chain Domain

```
            |---------- 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
```

Humanized 2H7.v16 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG._2

Humanized 2H7.v16 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

FIG._3

Humanized 2H7.v31 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

FIG._4

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._5

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._6

68

<110> GENENTECH, INC.
FROHNA, PAUL A.

<120> METHOD FOR TREATING MULTIPLE SCLEROSIS

<130> P2134R1 PCT

<140> PCT/US2005/019641
<141> 2005-06-02

<150> US 60/576,993
<151> 2004-06-04

<160> 25

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

<400> 1
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> 2
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 2
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> 3
<211> 108

69

<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 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> 4
<211> 10
<212> PRT
<213> Mus musculus

<400> 4
Arg Ala Ser Ser Ser Val Ser Tyr Met His
5 10

<210> 5
<211> 7
<212> PRT
<213> Mus musculus

<400> 5
Ala Pro Ser Asn Leu Ala Ser
5

<210> 6
<211> 9
<212> PRT
<213> Mus musculus

<400> 6
Gln Gln Trp Ser Phe Asn Pro Pro Thr
5

<210> 7
<211> 122
<212> PRT
<213> Mus musculus

<400> 7
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> 8
<211> 122
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 8
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> 9
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Sequence is synthesized.

<400> 9
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 Thr 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> 10
<211> 10
<212> PRT
<213> Mus musculus

<400> 10
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
5 10

<210> 11
<211> 17
<212> PRT
<213> Mus musculus

<400> 11
Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
1 5 10 15

Lys Gly

<210> 12
<211> 13
<212> PRT
<213> Mus musculus

<400> 12
Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
5 10

<210> 13
<211> 213
<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 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> 14
<211> 452

<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	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> 15
<211> 452
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 15
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 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

Lys 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> 16
<211> 213
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 16
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> 17
<211> 452
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<400> 17
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> 18
<211> 10
<212> PRT
<213> Artificial sequence

<220>
<223> sequence is synthesized

<220>
<221> Xaa
<222> 9
<223> Xaa is M or L

<400> 18
Arg Ala Ser Ser Ser Val Ser Tyr Xaa His
5 10

<210> 19
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<220>
<221> Xaa
<222> 4
<223> Xaa is S or A

<400> 19
Gln Gln Trp Xaa Phe Asn Pro Pro Thr
5

<210> 20
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<220>
<221> Xaa
<222> 8
<223> Xaa is D or A

<400> 20
Ala Ile Tyr Pro Gly Asn Gly Xaa Thr Ser Tyr Asn Gln Lys Phe
1 5 10 15

Lys Gly

77

<210> 21
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized.

<220>
<221> Xaa
<222> 6
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Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
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Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
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Asn Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser
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Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
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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
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Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
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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
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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
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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
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Ser Ser

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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

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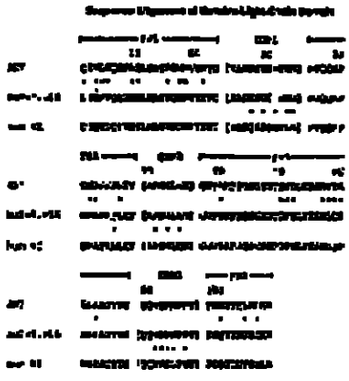
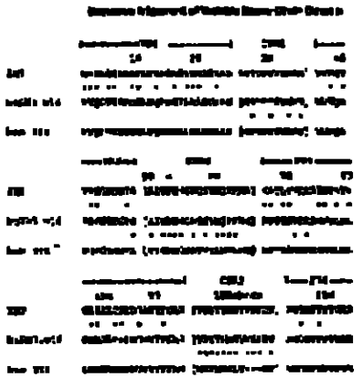
(54) Título: MÉTODO PARA TRATAR LA ESCLEROSIS MÚLTIPLE





(57) Resumen: Se describen métodos para tratar la esclerosis múltiple (EM) con un anticuerpo CD20 utilizando regímenes y protocolos de dosificación especial. También se describen artículos de manufactura para utilización en dichos métodos.

MÉTODO PARA TRATAR ESCLEROSIS MÚLTIPLE

Campo de la Invención

La presente invención se relaciona con métodos para tratar esclerosis múltiple (MS) en un sujeto utilizando regímenes y protocolos de dosificación especial, y un artículo de manufactura con instrucciones para tal uso.

Antecedentes de la Invención

Esclerosis Múltiple

La esclerosis múltiple (MS) es una enfermedad degenerativa inflamatoria y desmelanizante del sistema nervioso central humano (CNS) es una enfermedad mundial que afecta aproximadamente a 300,000 personas en los Estados Unidos; esta es una enfermedad de adultos jóvenes, con 70%-80% teniendo inicio entre los 20 y 40 años (Anderson *et al Ann Neurology* 31(3):333– 6 (1992); Noonan *et al Neurology* 58:136-8 (2002)). La MS es un desorden heterogéneo basado en el curso clínico, valoración de exploración de imágenes de resonancia magnética (MRI), y análisis de patología de biopsia y material de autopsia (Lucchinetti *et al. Ann Neurol* 47:707-17 (2000)). La enfermedad se manifiesta en si misma en un gran número de posibles combinaciones de déficit, incluyendo la columna vertebral, tronco encefálico, nervios craneales, cerebelosos, cerebrales, y síndromes cognitivos. La discapacidad

progresiva es el destino de la mayoría de los pacientes con MS, especialmente cuando se incluye una perspectiva de 25 años. La mitad de los pacientes con MS requieren un bastón para caminar 15 años a partir del inicio de la enfermedad. La MS es la causa principal de discapacidad neurológica en adultos jóvenes y de edad media y, hasta la década pasada no tenía tratamientos benéficos conocidos. La MS es difícil de diagnosticar en razón de los hallazgos clínicos no específicos, que conducen al desarrollo de criterios de diagnóstico altamente estructurados que incluyen varios avances tecnológicos, que consisten en exploraciones MRI, potenciales evocados, y estudios del fluido cerebro espinal (CSF). Todos los criterios de diagnóstico confían en los principios generales de lesiones esparcidas en la materia blanca central que ocurren en diferentes momentos y no explicadas por otras etiologías tales como infección, desorden vascular, o desorden autoinmune (McDonald *et al. Ann Neurol* 50:121-7 (2001)). La MS tiene cuatro patrones de enfermedad: MS recidivante-remitente (RRMS; 80%-85% de los casos al momento del inicio), MS progresiva primaria (PPMS; 10%– 15% en el momento del inicio), MS progresiva recidivante (PRMS; 5% al momento del inicio); y MS progresiva secundaria (SPMS) (Kremenchutzky *et al. Brain* 122 (Pt 10):1941– 50 (1999); Confavreux *et al N Engl J Med* 343(20): 1430-8 (2000)). Un 50% estimado de pacientes con RRMS desarrollará SPMS a los 10 años, y hasta el 90% de los pacientes RRMS eventualmente desarrollará SPMS (Weinshenker *et al. Brain* 112(Pt 1):133-6 (1989)).

Habitualmente, seis drogas en cuatro clases están aprobadas en los Estados Unidos para el tratamiento de RRMS, mientras que no se han aprobado drogas para PPMS. Los tratamientos de RRMS incluyen los siguientes: en la clase interferón, IFN-beta-1a (REBIF® y AVONEX®) e IFN-beta-1b (BETASERON®); glatiramer acetato (COPAXONE®), un polipéptido; natalizumab (TYSABRI®); y mitoxantrona (NOVANTRONA®), un agente citotóxico. Otras drogas se han utilizado con grados variantes de éxito, incluyendo corticosteroides, metotrexato, ciclofosfamida, azatioprina, e inmunoglobulina intravenosa (IV). Los beneficios de los tratamientos habitualmente o aprobados actualmente son relativamente modestos (~30%) para tasa de recaída y prevención de discapacidad en RRMS como lo sugirieron dos recientes meta-análisis (Filippini *et al. Lancet* 361: 545-52 (2003)).

Otros estudios clínicos evaluaron otros agentes inmunomodulatorios en MS, incluyendo inhibidores de factor α de necrosis tumoral y ligandos de péptido alterados, que agravaron en lugar de mejorar la MS (Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI *Neurology* 53:457-65 (1999); Bielekova *et al. Nat Med* 2000; 6: 1167-75 (2000), erratum appears en *Nat Med* 6: 1412 (2000)).

La visión predominante de la patología MS ha sostenido que la inflamación es principalmente mediada por células T $CD4^+$ Th1. Las aproximaciones terapéuticas basadas en esta teoría tales como IFN-beta y disminución del acetato de glatiramer, pero no evitan

completamente, la ocurrencia de exacerbaciones o acumulación de discapacidad.

La existencia del componente humoral en MS humana ha sido implícitamente reconocida durante décadas, como se evidencia mediante la inclusión de bandas oligoclonales CFS y la síntesis IgG intratecal incrementada en criterios de diagnóstico para la MS (Siden A. *J Neurol* 221:39-51(1979); McDonald *et al. Ann Neurol* 50:121-7 (2001); Andersson *et al. Eur J Neurol* 9:243-51 (2002); O'Connor, P. *Neurology* 59:S1-33 (2002)). La presencia de las bandas oligoclonales, cadenas ligeras libres incrementadas, y la síntesis IgM intratecal incrementada se correlaciona con la actividad de la enfermedad de MS y puede ser un elemento de predicción de ataques más severos (Rudick *et al. Mult Scler* 1:150-5 (1995); Zeman *et al. Acta Cytol* 45:51-9 (2001); Izquierdo *et al. Acta Neurol Scand* 105:158-63 (2002); Wolinsky J. *J Neurol Sci* 206:145-52 (2003); Villar *et al. Ann Neurol* 53:222-6 (2003)).

Los anticuerpos anti-mielina (proteína básica de mielina (MBP) y glicoproteína oligodendrocito mielina (MOG)) se han detectado en el suero de pacientes con formas progresivas y de recaída de la MS (Reindl *et al. Brain* 122:2047-56 (1999); Egg *et al. Mult Scler* 7(5):285-9 (2001)). También se han detectado anticuerpos anti-mielina en los pacientes CSF de MS (Reindl *et al. Brain* 122:2047-56 (1999); Egg *et al. Mult Scler* 7(5):285-9 (2001); Andersson *et al. Eur J Neurol* 9:243- 51 (2002)). Los tipos adicionales de anticuerpos tales como los anticuerpos anti-

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gangliosida o anticuerpos anti-neurofilamento se han observado en pacientes con la MS (Mata *et al. Mult Scler* 5:379-88 (1999); Sadatipour *et al. Ann Neurol* 44:980-3 (1998)). Un reporte reciente indicó que la presencia de anticuerpos anti-MOG y anti-MBP en el suero era un elemento de predicción fuerte de la progresión desde el evento desmelanizante clínicamente aislado para definir RRMS (Berger *et al. N Engl J Med* 349:139-45 (2003)). La tasa de riesgo ajustada para experimentar una exacerbación fue de 76.5 para pacientes que fueron seropositivos para ambos anticuerpos y 31.6 para pacientes que fueron seropositivos solamente para anti-MOG.

Un consorcio de patología internacional encontró que los anticuerpos encontrados en la mielina están presentes en la mayoría de los pacientes con MS, con células de plasma y células B también encontradas en lesiones MS, que suministran evidencia adicional para un papel humoral en la MS (Prineas y Wright, *Lab Invest* 38:409-21 (1978); Esiri M. *Neuropathol Appl Neurobiol* 6:9-21 (1980); Genain *et al. Nat Med* 5:170-5 (1999); Lucchinetti *et al. Ann Neurol* 47:707-17 (2000); Wingerchuk *et al. Lab Invest* 81: 263-81 (2001)). Las células B son detectables en el CSF de pacientes con MS, y la presencia de una proporción relativamente alta de células B puede ser predictiva de una progresión de la discapacidad más severa (Cepok *et al. Brain* 124(Pt 11):2169-76 (2001)).

En sujetos con RRMS o síndrome de opsoclonus-mioclonus, el Rituximab reportó el vaciado de células B periféricas en todos los sujetos y disminuyo el número de células B CSF en algunos pacientes (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003); Cross *et al.* "Preliminary Results from a Phase II Trial of Rituximab in MS" (abstract) *Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis ACTRIMS* 20-1 (Octubre, 2003)). Ver también Cree *et al.* "Tolerability and Effects of Rituximab "Anti-CD20 Antibody" en Neuromyelitis Optica and Rapidly Worsening Multiple Sclerosis" *Meeting of the Am. Acad. Neurol.* (Abril, 2004).

Anticuerpos CD20 y su Terapia

Los linfocitos son uno de los muchos tipos de células sanguíneas blancas producidas en la médula ósea durante el proceso de la hematopoyesis. Existen dos poblaciones principales de linfocitos; los linfocitos B (células B) y los linfocitos T (células T). Los linfocitos de interés particular aquí son las células B.

Las células B maduran dentro de la médula ósea y dejan la médula expresando un anticuerpo de unión de antígeno sobre su superficie celular. Cuando una célula B no diferenciada encuentra primero el antígeno para el cual su anticuerpo de unión de membrana es específico, la célula inicia su división rápidamente y su progenie se diferencia en células B de memoria y células

efectuadoras denominadas "células de plasma". Las células B memoria tienen un lapso de vida más largo y continúan expresando anticuerpos de unión de membrana con la misma especificidad que la célula padre original. Las células de plasma no producen anticuerpo de unión de membrana pero en su lugar producen el anticuerpo en una forma que puede ser secretada. Los anticuerpos secretados son la molécula efectuadora principal de la inmunidad humoral.

El antígeno CD20 (también denominado antígeno de diferenciación restringido de linfocito B humano, Bp35) es una proteína de transmembrana hidrófoba con un peso molecular de aproximadamente 35 kD es localizado sobre lo linfocitos pre-B y B maduros (Valentine *et al. J. Biol. Chem.* 264(19): 11282-11287 (1989); y Einfeld *et al. EMBOJ.* 7(3):711-717 (1988)). El antígeno también se expresa en más del 90% de los linfomas no Hodking de célula B (NHL) (Anderson *et al. Blood* 63(6):1424-1433 (1984)), pero no se encuentra en las células madre hematopoyéticas, células procedimiento-B, células de plasma normales u otros tejidos normales (Tedder *et al. J. Immunol.* 135(2):973-979 (1985)). El CD20 regula el o las etapas iniciales del proceso de activación para la iniciación del ciclo de la célula y la diferenciación (Tedder *et al., supra*) y posiblemente funciona como el canal de ión-calcio (Tedder *et al. J. Cell. Biochem.* 14D:195 (1990)).

Dada la expresión del CD20 en linfomas de célula B, este antígeno puede servir como un candidato para el "destino" de tales linfomas. En esencia, tal destino se puede generalizar como sigue: anticuerpos específicos para el antígeno de superficie CD20 de células B se administran a un paciente. Estos anticuerpos CD20 se unen específicamente al antígeno CD20 de (ostensiblemente) tanto células B normales como malignas; la unión del anticuerpo al antígeno de superficie CD20 puede conducir a la destrucción y vaciamiento de células B neoplásicas. Adicionalmente, los agentes químicos o los marcadores radioactivos que tienen el potencial de destruir el tumor se pueden conjugar al anticuerpo anti-CD20 de tal forma que el agente es específicamente "suministrado" a las células B neoplásicas. Sin importar la aproximación, la primera meta es destruir el tumor; la aproximación específica se puede determinar mediante el anticuerpo anti-CD20 particular que se utiliza y, así, las aproximaciones disponibles para apuntar al antígeno CD20 pueden variar considerablemente.

El anticuerpo Rituximab (RITUXAN®) es un anticuerpo monoclonal de murino/humano quimérico genéticamente manejado con ingeniería dirigido contra el antígeno CD20. El Rituximab es el anticuerpo denominado "C2B8" en la Patente US No. 5,736,137 otorgada en Abril 7, 1998 (Anderson et al). El RITUXAN® se indica para tratamiento de pacientes con linfoma no Hodking de célula B CD20 positivo de bajo grado o folicular recaído o refractario. El mecanismo *in vitro* de los estudios de acción han demostrado que

el RITUXAN® se une al complemento humano y lisa líneas de célula B linfoides a través de citotoxicidad dependiente de complemento (CDC) (Reff *et al. Blood* 83(2):435-445 (1994)). Adicionalmente, tiene una actividad significativa en ensayos para citotoxicidad celular dependiente de anticuerpo (ADCC). Más recientemente, el RITUXAN® ha mostrado tener efectos anti-proliferativos en ensayos de incorporación de timidina tritiada y par inducir apoptosis directamente, mientras que otros anticuerpos anti-CD19 y CD20 no (Maloney *et al. Blood* 88(10):637a (1996)). La sinergia entre RITUXAN® y quimioterapias y toxinas también se ha observado experimentalmente. En particular, el RITUXAN® sintetiza líneas de célula de linfoma de célula B humana resistente a las drogas a los efectos citotóxicos de doxorubicina, CDDP, VP-16, toxina de difteria y ricina (Demidem *et al. Cancer Chemotherapy & Radiopharmaceuticals* 12(3):177-186 (1997)). Los estudios preclínicos *in vivo* han mostrado que el RITUXAN® vacía las células B de la sangre periférica, los nodos linfáticos, y la médula ósea de monos cinomolgus, presumiblemente a través de procesos mediados por complemento y célula (Reff *et al. Blood* 83(2):435-445 (1994)).

El rituximab se aprobó en los Estados Unidos en noviembre de 1997 para el tratamiento de pacientes con linfoma no Hodking de célula B $CD20^+$ de bajo grado o folicular recaído o refractario a una dosis de 375 mg/m^2 semanalmente para cuatro dosis. En Abril de 2001, la administración para alimentos y drogas (FDA) aprobó reivindicaciones adicionales para el tratamiento de NHL de bajo

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grado: retratamiento (semanalmente para cuatro dosis) y un régimen de dosificación adicional (semanalmente para 8 dosis). Ha habido más de 300,000 pacientes expuestos al Rituximab como monoterapia o en combinación con drogas inmunosupresoras o quimioterapéuticas. Los pacientes también han sido tratados con Rituximab como terapia de mantenimiento durante hasta 2 años (Hainsworth *et al. J Clin Oncol* 21:1746-51 (2003); Hainsworth *et al. J Clin Oncol* 20:4261-7 (2002)).

El Rituximab también ha sido estudiado en una variedad de desórdenes autoinmunes no malignos, en los cuales las células B y los anticuerpos parecen jugar un papel en la patofisiología de la enfermedad (Edwards *et al. Biochem Soc Trans* 30:824-8 (2002)). El Rituximab se ha reportado por aliviar potencialmente los signos y los síntomas de la artritis reumatoide (RA) (Leandro *et al. Ann Rheum Dis.* 61:883-8 (2002); Emery *et al. Arthritis Rheum* 48(9): S439 (2003)), lupus (Eisenberg R. *Arthritis Res Ther* 5:157-9 (2003); Leandro *et al. Arthritis Rheum* 46:2673-7 (2002)), trombocitopenia inmune (D'Arena *et al. Leuk Lymphoma* 44:561-2 (2003)), anemia autoinmune (Zaja *et al. Haematologica* 87:189-95 (2002) (erratum appears in *Haematologica* 87:336 (2002)), neuropatía autoinmune (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485-9 (2003)), síndrome opsoclonus-mioclonus paraneoplásico (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), y esclerosis múltiple recidivante-remisión (RRMS) (Cross et al. (abstract) Eighth Annual Meeting of the

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Americas Committees for Research and Treatment in Multiple Sclerosis 20-1 (2003)).

Un estudio de fase II (WA16291) ha sido conducido en pacientes con artritis reumatoide (RA), que suministra datos de seguimiento de 48 semanas sobre la seguridad y eficacia del Rituximab (Emery *et al. Arthritis Rheum* 48(9):S439 (2003); Szczepanski *et al. Arthritis Rheum* 48(9):S121 (2003)). Un total de 161 pacientes fueron homogéneamente aleatorizados para cuatro brazos de tratamiento: metotrexato, Rituximab solo, Rituximab más metotrexato, Rituximab más ciclofosfamida (CTX). El régimen de tratamiento de Rituximab fue de 1 g administrado intravenosamente en los Días 1 y 15. Las infusiones de Rituximab en la mayoría de los pacientes con RA fueron bien tolerados por la mayoría de los pacientes, con 36% de los pacientes experimentando al menos un evento adverso durante su primer infusión (comparado con el 30% de los pacientes que reciben placebo). En total, la mayoría de los eventos adversos fueron considerados como medios a moderados en severidad y fueron bien balanceados en todos los grupos de tratamiento. Hubo un total de 19 eventos adversos serios en los cuatro brazos durante las 48 semanas, que fueron ligeramente más frecuentes en el grupo Rituximab/CTX. La incidencia de infecciones estuvo bien balanceada en todos los grupos. La tasa media de infección seria en esta población de pacientes RA fue de 4.6 6 por 100 pacientes-años, que es inferior que la tasa de infecciones que requiere admisión en el hospital en pacientes RA (9.57 por 100 pacientes-años) reportado en un estudio

epidemiológico basado en comunidades (Doran *et al. Arthritis Rheum* 46:2287-93 (2002)).

El perfil de seguridad reportado del Rituximab en un número pequeño de pacientes con desórdenes neurológicos, que incluyen neuropatía autoinmune (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485-9 (2003)), síndrome opsoclonus/mioclonus (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), y RRMS (Cross *et al.* Preliminary results from a phase II trial of Rituximab in MS (abstract) Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis 20-1 (2003)), fue similar a aquel reportado en oncología o RA. En un ensayo con patrocinio de los investigadores en proceso (IST) de Rituximab en combinación con interferón-beta (IFN-beta) o acetato de glatiramer en sujetos con RRMS (Cross *et al., supra*), 1 de 10 sujetos tratados se admitió en hospital para observación durante toda la noche después de experimentar fiebre moderada y los rigores que siguen a la primer infusión de Rituximab, aunque los otros 9 sujetos completaron el régimen de cuatro infusiones sin ningún evento adverso reportado.

Las patentes y publicaciones de patentes con relación a los anticuerpos CD20 incluyen las Patentes Estadounidenses Nos. 5,776,456, 5,736,137, 5,843,439, 6,399,061, y 6,682,734, así como también la US 2002/0197255, US 2003/0021781, US 2003/0082172, US 2003/0095963, US 2003/0147885 (Anderson *et al.*); la Patente Estadounidense No 6,455,043, US 2003/0026804 y WO 2000/09160

(Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez y White); WO 2000/27433 y US 2004/0213784 (Grillo-Lopez y Leonard); WO 2000/44788 (Braslawsky et al); WO 2001/10462 (Rastetter, W.); WO01/10461 (Rastetter y White); WO 2001/10460 (White y Grillo-Lopez); US 2001/0018041, US 2003/0180292, WO 2001/34194 (Hanna y Hariharan); US 2002/0006404 y WO 2002/04021 (Hanna y Hariharan); US 2002/0012665 y WO 2001/74388 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan y Hanna); US 2002/0009444 y WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858 (White, C); US 2002/0128488 y WO 2002/34790 (Reff, M.); WO 2002/060955 (Braslawsky *et al.*);WO 2002/096948 (Braslawsky *et al.*);WO 2002/079255 (Reff y Davies); la Patente US No. 6,171,586 y WO 1998/56418 (Lam *et al*); WO 1998/58964 (Raju, S.); WO 1999/22764 (Raju, S.); WO 1999/51642, la Patente US No. 6,194,551, la Patente US No. 6,242,195, la Patente US No. 6,528,624 y la Patente US No. 6,538,124 (Idusogie *et al*); WO 2000/42072 (Presta, L.); WO 2000/67796 (Curd *et al.*); WO 2001/03734 (Grillo-Lopez *et al.*); US 2002/0004587 y WO 2001/77342 (Miller y Presta); US 2002/0197256 (Grewal, I); US 2003/0157108 (Presta, L.); WO 04/056312 (Lowman *et al*); US 2004/0202658 y WO 2004/091657 (Benyunes, K.); WO 2005/000351 (Chan, A.); US 2005/0032130A1 (Beresini *et al*); US 2005/0053602A1 (Brunetta, P.); las Patentes US Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, y 5,595,721, (Kaminski *et al*); las Patentes US Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, y 6,652,852 (Robinson *et al*); Patente US No. 6,410,391 (Raubitschek *et al*); la Patente US No. 6,224,866 y WO00/20864 (Barbera-Guillem, E.); WO 2001/13945

(Barbera-Guillem, E.); US2005/0079174A1 (Barbera-Guillem *et al*); WO 2000/67795 (Goldenberg); US 2003/0133930 y WO 2000/74718 (Goldenberg y Hansen); US 2003/0219433 y WO 2003/68821 (Hansen *et al.*); WO2004/058298 (Goldenberg y Hansen); WO 2000/76542 (Golay *et al*);WO 2001/72333 (Wolin y Rosenblatt); la Patente US No. 6,368,596 (Ghetie *et al*); la Patente US No. 6,306,393 y US 2002/0041847 (Goldenberg, D.); US 2003/0026801 (Weiner y Hartmann); WO 2002/102312 (Engleman, E.); US 2003/0068664 (Albitar *et al*); WO 2003/002607 (Leung, S.); WO 2003/049694, US2002/0009427, y US 2003/0185796 (Wolin *et al*); WO 2003/061694 (Sing y Siegall); US 2003/0219818 (Bohen *et al*); US 2003/0219433 y WO 2003/068821 (Hansen *et al*); US 2003/0219818 (Bohen *et al*); US2002/0136719 (Shenoy *et al*); WO 2004/032828 (Wahl *et al*); y WO 2002/56910 (Hayden-Ledbetter). Ver también la Patente US No. 5,849,898 y EP 330,191 (Seed *et al.*); EP332,865A2 (Meyer y Weiss); la Patente US No. 4,861,579 (Meyer *et al*); US2001/0056066 (Bugelski *et al*); WO 1995/03770 (Bhat *et al*); US 2003/0219433 A1 (Hansen *et al*); WO 2004/035607 (Teeling *et al*); US 2004/0093621 (Shitara *et al*); WO 2004/103404 (Watkins *et al*); WO 2005/000901 (Tedder *et al*); US 2005/0025764 (Watkins *et al*); WO2005/016969 y US 2005/0069545 A1 (Carr *et al*); WO 2005/014618 (Chang *et al*). Ciertas de estas incluyen, *inter alia*, tratamiento de esclerosis múltiple.

Las publicaciones que se relacionan con la terapia de Rituximab incluyen: Perotta and Abuel "Response of chronic relapsing ITP of 10 years duration to Rituximab" Abstract # 3360

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Blood 10(1) (parte 1-2): p. 88#-(1998); Stashi *et al.* "Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idopathic thrombocytopenic purpura" *Blood* 98(4):952-957 (2001); Matthews, R. "Medical Heretics" *New Scientist* (7 Abril, 2001); Leandro *et al.* "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" *Ann Rheum Dis* 61: 833-888 (2002); Leandro *et al.* "Lymphocyte depletion in rheumatoid arthritis: early evidence for safety, efficacy and dose response". *Arthritis and Rheumatism* 44(9): S370 (2001); Leandro *et al.* "An open study of B lymphocyte depletion in systemic lupus erythematosus", *Arthritis & Rheumatism* 46(1):2673-2677 (2002); Edwards and Cambridge "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rhematology* 40:205-211 (2001); Edwards *et al.* "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem. Soc. Trans.* 30(4):824-828 (2002); Edwards *et al.* "Efficacy and safety of Rituximab, a B-cell targeted chimeric monoclonal antibody: A randomized, placebo controlled trial in patients with rheumatoid artritis". *Arthritis and Rheumatism* 46(9): S 197 (2002); Levine and Pestronk "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab" *Neurology* 52: 1701-1704 (1999); DeVita *et al.* "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hidashida *et al.* "Treatment of DMARD-Refractory rheumatoid arthritis with Rituximab." Presentado en el *Annual Scientific Meeting of the American College of Rheumatology;* Oct

24-29; New Orleans, LA 2002; Tuscano, J. "Successful treatment of Infliximab-refractory rheumatoid arthritis with Rituximab" Presentado en el *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001); Anolik *et al.*, "B lympocyte Depletion in the Treatment of Systemic Lupus (SLE): Phase I/II Trial of Rituximab (RITUXAN®) in SLE" *Arthritis And Rheumatism*, 46(9), S289-S289 Abstract 717 (Octubre, 2002), y Albert *et al.*, "A Phase I Trial of Rituximab (Anti-CD20) for Treatment of Systemic Lupus Erythematosus" *Arthritis And Rheumatism*, 48(12): 3659-3659, Abstract LB9 (Diciembre, 2003); Martin and Chan "Pathogenic Roles of B cells in Human Autoimmunity: Insights from the Clinic" *Immunity* 20:517-527 (2004).

Resumen de la Invención

La presente invención involucra, al menos en parte, la selección de una dosis para un anticuerpo CD20 que suministra un régimen de tratamiento seguro y activo en sujetos con esclerosis múltiple, tal como PPMS o RRMS.

De acuerdo con esto, la invención se relaciona con un método para tratar esclerosis múltiple en un sujeto que comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto para suministrar un exposición de anticuerpo inicial de

aproximadamente 0.5 a 4 gramos seguida por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se suministra hasta aproximadamente 16 a 60 semanas de la exposición inicial, y cada uno de las exposiciones a anticuerpo se suministra al sujeto como una o dos dosis de anticuerpo.

Además, la invención se relaciona con un artículo de manufactura que comprende: (a) un recipiente que comprende un anticuerpo CD20; y (b) un inserto de paquete con instrucciones para tratar esclerosis múltiple en un sujeto, en donde las instrucciones indican que la cantidad del anticuerpo se administra al sujeto que es efectiva para suministrar una exposición inicial al anticuerpo de aproximadamente 0.5 a 4 gramos seguida por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se administrada hasta las 16 a 60 semanas desde la exposición inicial, y cada una de las exposiciones a anticuerpo se suministra al sujeto como una o dos dosis de anticuerpo.

Breve Descripción de los Dibujos

La figura 1A es un alineamiento de secuencia que compara a las secuencias de aminoácido del dominio variable de cadena ligera (V_L) de cada murino 2H7 (SEQ ID NO:1), variante 2H7.v16 humanizada (SEQ ID NO:2), y subgrupo I de cadena ligera kappa humano (SEQ ID NO:3). Los CDR de V_L de 2H7 y hu2H7.v16 son como

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sigue: CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:5), y CDR3 (SEQ ID NO:6).

La figura 1B es un alineamiento de secuencia que compara a las secuencias de aminoácido del dominio variable de cadena pesada (V_H) de cada murino 2H7 (SEQ ID NO:7), la variante 2H7.v16 humanizada (SEQ ID NO:8), y la secuencia de consenso humano del subgrupo III de cadena pesada (SEQ ID NO:9). Los CDR de V_H de 2H7 y hu2H7.v16 son como sigue: CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO: 11), y CDR3 (SEQ ID NO: 12).

En la figura 1A y la figura 1B, el CDR1, CDR2 y CDR3 en cada cadena están incluidos dentro de corchetes, flanqueados por regiones marco, FR1-FR4, como se indica. 2H7 se refiere al anticuerpo 2H7 de murino. Los asteriscos entre las dos líneas de secuencias indican la posición que son diferentes entre las dos secuencias. La numeración del residuo es de acuerdo a Kabat *et al. Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), con inserciones mostradas como a, b, c, d, y e.

La figura 2 muestra la secuencia de aminoácido de la cadena ligera 2H7.v16 madura (SEQ ID NO:13).

La figura 3 muestra la secuencia de aminoácido de la cadena pesada 2H7.v16 madura (SEQ ID NO: 14).

La figura 4 muestra la secuencia de aminoácido de la cadena pesada 2H7.v31 madura (SEQ ID NO:15).
La cadena L del 2H7.v31 es la misma que para el 2H7.v16.

La figura 5 muestra un alineamiento de las cadenas ligera 2H7.v16 y 2H7.v511 madura (SEQ ID NOS. 13 y 16, respectivamente), con una numeración de residuo de dominio variable Kabat y una numeración de residuo constante Eu.

La figura 6 muestra un alineamiento de las cadenas pesadas 2H7.v16 y 2H7.v511 maduras (SEQ ID NOS. 14 y 17, respectivamente), con una numeración de residuo de dominio variable Kabat y una numeración de residuo constante Eu.

Descripción Detallada de las Modalidades Preferidas

I. Definiciones

Una "célula B" es un linfocito que madura dentro de la médula ósea, e incluye una célula B no diferenciada, célula B memoria, o célula b efectuadora (células de plasma). La célula B aquí puede ser una célula b normal o no maligna.

Un "marcador de superficie de célula B" o "antígeno de superficie de célula B" aquí es un antígeno expresado sobre la superficie de la célula B que se puede ser blanco con un anticuerpo que se une a este. Marcadores de superficie de célula

B de ejemplo incluyen el 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 marcadores de superficie de leucocito (para las descripciones, ver The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Otros marcadores de superficie de célula B incluyen RP105, FcRH2, CR2 de célula 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 célula B de interés particular aquí es predominantemente expresado sobre células B comparado con otros tejidos de célula no B de mamífero y se puede expresar tanto en las células B precursoras como en las células B maduras. El marcado preferido de superficie de célula B es CD20.

El "antígeno CD20" o "CD20" es una fosfoproteína no glicosilada de aproximadamente 35-kDa encontrada en la superficie de la mayoría de más del 90% de las células B proveniente de la sangre periférica o de órganos linfoides. El CD20 está presente tanto en células B normales así como también en células B malignas, pero no se expresa sobre células madre. Otros nombres para CD20 en la literatura incluyen "antígeno restringido de linfocito B" y "Bp35". El antígeno CD20 se describe en Clark *et al. Proc. Natl. Acad. Sci* (USA) 82: 1766 (1985), por ejemplo.

Un "antagonista de anticuerpo" aquí es un anticuerpo que, luego de la unión al marcador de superficie de célula B sobre las

células B, destruye o vacía las células B en un mamífero y/o interfiere con una o más funciones de la célula B, por ejemplo, al reducir o prevenir una respuesta tumoral elicitada por la célula B. El antagonista de anticuerpo preferiblemente es capaz de vaciar las células B (es decir reducir los niveles de célula B circulantes) en un mamífero tratado con este. Tal vaciamiento se puede lograr por vía de varios de mecanismos tales como citotoxicidad mediada por célula dependiente de anticuerpo (ADCC) y/o y citotoxicidad dependiente de complemento (CDC), inhibición de proliferación de célula B y/o inducción de muerte de célula B (por ejemplo vía apoptosis).

"Citotoxicidad mediada por célula dependiente de anticuerpo" y "ADCC" se refieren a la reacción mediada por célula en la cual las células citotóxicas no específicas que expresan receptores Fc (FcRs) (por ejemplo Células Asesinas Naturales (NK), neutrófilos, y macrófagos) reconocen el anticuerpo de unión sobre una célula blanco y subsecuentemente causan la lisis de la célula blanco. Las células primarias para mediar ADCC, células NK, expresan solamente FcγRIII, mientras que los monocitos expresan FcγRI, FcγRII y FcγRIII. La expresión de FcR sobre las células hematopoyéticas se resumen en la Tabla 3 en la página 464 de Ravetch y Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). Para evaluar la actividad ADCC de una molécula de interés, un ensayo ADCC *in vitro*, tal como aquel descrito en la Patente US No. 5,500,362 o 5,821,337 se puede desarrollar. Las células efectuadoras útiles para tales ensayos incluyen células mononucleares de sangre

periférica (PBMC) y células asesinas naturales (NK). Alternativamente, o adicionalmente, la actividad ADCC de la molécula de interés se puede valuar *in vivo*, por ejemplo, en un modelo animal tal como aquel descrito en Clynes *et al. PNAS* (USA) 95:652-656 (1998).

"Células efectuadoras humanas" son leucocitos que expresan uno o más FcRs y desarrollan funciones efectuadoras. Preferiblemente, las células expresan al menos FcγRIII y llevan a cabo función efectuadora ADCC. Ejemplos de leucocitos humanos que median ADCC incluyen células mononucleares de sangre periférica (PBMC), células asesinas naturales (NK), células T citotóxicas y neutrófilos; con los PBMC y las células NK siendo las preferidas.

Los términos "receptor Fc" o "FcR" se utilizan para describir un receptor que se une a la región Fc de un anticuerpo. El FcR preferido es un FcR humano de secuencia nativa, aún, un FcR preferido es aquel que se une al anticuerpo IgG (un receptor gama) e incluye los receptores de las subclases FcγRI, FcγRII y FcγRIII, que incluyen variantes alélicas y formas alternativamente empalmadas de estos receptores. Los receptores FcγRII incluyen FcγRIIA (un "receptor activante") y FcγRIIB (un "receptor de inhibición"), que tiene secuencias de aminoácido similares que difieren primariamente en los dominios citoplasmáticos de estos. El receptor activante FcγRIIA contiene un motivo de activación basado en tirosina inmunoreceptora (ITAM)

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en su dominio citoplasmático. El receptor de inhibición FcγRIIB contiene un motivo de inhibición basado en tirosina inmunoreceptora (ITIM) y su dominio citoplasmático. (Ver Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). Los FcRs son recibidos 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, que incluyen aquellos a ser identificados en el futuro, están comprendidos en el término "FcR" aquí. El término también incluye receptor neonatal, FcRn, que es responsable por la transferencia de los IgG maternos a los fetos (Guyer *et al., J. Immunol.* 117:587 (1976) y Kim *et al., J. Immunol.* 24:249 (1994)).

"La citotoxicidad dependiente de complemento" o "CDC" se refiere a la capacidad de una molécula a lisar un blanco en la presencia de complemento. La senda de activación de complemento se inicia mediante la unión del primer componente del sistema componente (C1q) a una molécula (por ejemplo un anticuerpo) complejado con un antígeno cognado. Para evaluar la activación de complemento, un ensayo CDC, por ejemplo, como se describe en Gazzano-Santoro *et al., J. Immunol. Methods* 202: 163 (1996), se puede desarrollar.

Los anticuerpos de "crecimiento inhibitorio" son aquellos que evitan o reducen la proliferación de una célula que expresa un antígeno al cual se une el anticuerpo. Por ejemplo, el

anticuerpo puede prevenir o reducir la proliferación de células B *in vitro* y/o *in vivo*.

Los anticuerpos que "inducen apoptosis" son aquellos que inducen la muerte de célula programada, por ejemplo de una célula B, como se determina mediante ensayos de apoptosis estándar, tales como unión de anexina V, fragmentación de ADN, encogimiento de célula, dilatación de retículo endoplasmático, fragmentación de célula, y/o formación de vesículas de membrana (denominadas cuerpos apoptóticos).

El término anticuerpo aquí se utiliza en el sentido más amplio y cubre específicamente anticuerpos monoclonales, anticuerpos policlonales, anticuerpos multiespecíficos (por ejemplo anticuerpos biespecíficos) formados de al menos dos anticuerpos intactos, y fragmentos de anticuerpo en tanto ellos exhiban su actividad biológica deseada.

"Fragmentos de anticuerpo" comprende una porción de un anticuerpo intacto, preferiblemente que comprende la región de unión de antígeno de este. Ejemplos de fragmento de anticuerpo incluyen los fragmentos Fab, Fab', $F(ab')_2$, y Fv; diacuerpos; anticuerpos lineales; moléculas de anticuerpo de cadena simple; y anticuerpos multiespecíficos formados de fragmentos de anticuerpo.

Para los propósitos presentes, un "anticuerpo intacto" es uno que comprende dominios variables pesados y ligeros así como también una región Fc.

"Anticuerpos nativos" son usualmente glicoproteínas heterotetraméricas de aproximadamente 150,000 daltons, compuestos de cadenas ligeras idénticas (L) y dos cadenas pesadas idénticas (H). Cada cadena ligera está ligada a una cadena pesada a una unión disulfuro covalente, aunque el número de enlaces disulfuro varía entre las cadenas pesadas de diferentes isotipos de inmunoglobulina diferentes. Cada cadena pesada y ligera ha espaciado regularmente puentes disulfuro intracadena. 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 cadena ligera se alinea con el dominio variable de la cadena pesada. Los residuos de aminoácido particulares se cree que 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 ante los anticuerpos y son utilizados en la unión y especificidad de cada anticuerpo particular para su antígeno particular. Sin embargo, la variabilidad no es homogéneamente

distribuida a través de los dominios variables de anticuerpos. Se concentra en tres segmentos denominados regiones hipervariables tanto en los dominios de variable de cadena ligera como de cadena pesada. Las porciones más altamente conservadas de los dominios variables son denominadas regiones marco (FR). El dominio variable de las cadenas pesada y ligera nativas comprende cuatro FR, que adoptan grandemente la configuración de lámina β, conectada por tres regiones hipervariables, que forman bucles que conectan y en algunos casos forman parte de, la estructura de la lámina β. Las regiones hipervariables en cada cadena son mantenidas juntas en proximidad cercana mediante los FR y, con las regiones hipervariables de la otra cadena, contribuyen a la formación del sitio de unión de antígeno de los anticuerpos (ver 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 involucrados directamente en la unión de un anticuerpo a un antígeno, sino que exhiben varias funciones efectuadoras, tales como la participación del anticuerpo en citotoxicidad celular dependiente de anticuerpo (ADCC).

La digestión de la papaína de los anticuerpos produce dos fragmento de unión de antígeno idénticos, denominados fragmentos "Fab", cada uno con un sitio de unión de antígeno simple, y un fragmento "Fc" residual, cuyo nombre refleja su capacidad a cristalizar fácilmente. El tratamiento de pepsina produce un

fragmento $F(ab')_2$ que tiene dos sitios de unión de antígeno y es aún capaz de reticular antígeno.

"Fv" es el fragmento de anticuerpo mínimo que contiene un reconocimiento de antígeno completo y un sitio de unión de antígeno. Esta región consiste de un dímero de una cadena pesada y un dominio variable de cadena ligera en asocio no covalente estrecho. Es en esta configuración que las tres regiones hipervariables de cada dominio variable interactúan para definir el sitio de unión de antígeno sobre la superficie del dímero V_H-V_L. Colectivamente, las seis regiones hipervariables confieren especificidad de unión de antígeno al anticuerpo. Sin embargo, aún un dominio variable simple (o la mitad de un Fv que comprende solamente tres regiones hipervariables específicas para un antígeno) tiene la capacidad de reconocer y unir antígeno, aunque a una afinidad inferior que el sitio de unión completo.

El fragmento Fab también contiene un dominio constante de la cadena ligera y el primer dominio constante (CH1) de la cadena pesada. Los fragmentos Fab' difieren de los fragmentos Fab mediante la adición de unos pocos residuos del terminal carboxi del dominio CH1 de cadena pesada que incluye una o más cisteínas provenientes de la región pivote de anticuerpo. El Fab'-SH es la designación aquí para el Fab' en el cual el o los residuos de cisteína de los dominios constantes llevan al menos un grupo tiol libre. Los fragmentos de anticuerpo $F(ab')_2$ originalmente fueron producidos como pares de fragmentos Fab' que tienen cisteínas

pivote entre ellos. Otros acoplamientos químicos de fragmento de anticuerpo también son conocidos.

Las "cadenas ligeras" de anticuerpos (inmunoglobulinas) provenientes de cualquiera especies de vertebrados pueden ser asignadas a uno de dos tipos claramente distintos, denominados kappa (κ) y lambda (λ), con base en la secuencia de aminoácido de sus dominios constantes.

Dependiendo de la secuencia de aminoácido del dominio constante de sus cadenas pesadas, los anticuerpos se pueden asignar a diferentes clases. Existen clases principales de anticuerpos intactos: IgA, IgD, IgE, IgG, e IgM, y varios de estos pueden ser adicionalmente divididos 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 anticuerpo son denominados α, δ, ε, γ, y μ, respectivamente. Las estructuras subunitarias y las configuraciones tridimensionales de las diferentes clases de inmunoglobulina son bien conocidas.

Los fragmentos de anticuerpo "Fv de cadena simple" o "scFv" comprende los dominios V_H y V_L del anticuerpo, en donde estos dominios están presentes en una cadena de polipéptido simple. Preferiblemente, el polipéptido Fv comprende además un ligador de polipéptido entre los dominios V_H y V_L que posibilitan que el scFv forme la estructura deseada para la unión de antígeno. Para

una revisión del scFv ver Plückthun en *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg y Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

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

El término "anticuerpo monoclonal" como se utiliza aquí se refiere a un anticuerpo obtenido de una población de anticuerpos sustancialmente homogénea, es decir los anticuerpos individuales que comprenden la población son idénticos y/o se unen al mismo epítopo, excepto para posibles variantes que puedan surgir durante la producción del anticuerpo monoclonal, tales variantes están generalmente presentes en cantidades menores. Como contraste a las preparaciones de anticuerpo monoclonal que incluyen típicamente diferentes anticuerpos dirigidos contra diferentes determinantes (epítopos), cada anticuerpo monoclonal está dirigido contra un determinante simple sobre el antígeno.

Además de su especificidad, los anticuerpos monoclonales son ventajosos porque ellos no están contaminados por otras inmunoglobulinas. El modificador "monoclonal" indica el carácter del anticuerpo que está siendo obtenido de la población de anticuerpos sustancialmente homogénea, y no debe ser construido para requerir producción del anticuerpo mediante cualquier método particular. Por ejemplo, los anticuerpos monoclonales a ser utilizados de acuerdo con la presente invención se pueden hacer mediante el método de hibridoma primero descrito por Kohler *et al., Nature*, 256:495 (1975), o se puede hacer mediante método de ADN recombinante (ver, por ejemplo, Patente U.S: No. 4,816,567). Los "anticuerpos monoclonales" también se pueden aislar de las librerías de anticuerpo de fago que utilizan las técnicas descritas en Clackson *et al., Nature*, 352:624-628 (1991) y Marks *et al., J. Mol. Biol.*, 222:581-597 (1991), por ejemplo.

Los anticuerpos monoclonales aquí incluyen específicamente anticuerpos "quiméricos" (inmunoglobulina) en las cuales una porción de la cadena pesada y/o ligera es idéntica con un homóloga a secuencias correspondientes en anticuerpos derivados de especies particulares o que pertenecen a una clase o subclase de anticuerpo particular, aunque el resto de la o las cadenas es idéntico con u homólogo a las secuencias correspondientes en anticuerpos derivados de otras especies o que pertenecen a otra clase o subclase de anticuerpo, así como también fragmentos de tales anticuerpos en tanto que ellas exhiban la actividad biológica deseada (Patente U.S. No. 4,816,567; Morrison *et al.*,

Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). Los anticuerpos quiméricos de interés incluyen anticuerpos "primatizados" que comprenden secuencias de unión de antígeno de dominio variable derivadas de primate no humano (por ejemplo Mono del Viejo Continente, tales como mandril, rhesus o el mono cinomolgus) y secuencias de región constante humana (Patente Estadounidense No. 5,693,780).

Las formas "humanizadas" de anticuerpos no humanos (por ejemplo murino) son anticuerpos quiméricos que contienen secuencias mínimas derivadas de inmunoglobulina no humana. Para la mayor parte, los anticuerpos humanizados son inmunoglobulinas humanas (anticuerpo receptor) en las cuales los residuos provenientes de una región hipervariable del receptor son reemplazados por residuos de una región hipervariable de especies no humanas (anticuerpo donador) tales como ratón, rata, conejo, o primate no humano que tiene la especificidad deseada, afinidad, y capacidad. En algunos casos, los residuos de región de estructura (Fr) de la inmunoglobulina humana son reemplazados por los residuos no humanos correspondientes. Adicionalmente, los anticuerpos humanizados pueden comprender residuos que no se encuentran en el anticuerpo receptor o en el anticuerpo donador. Estas modificaciones son hechas para refinar además el desempeño del anticuerpo. En general, el anticuerpo humanizado comprenderá sustancialmente todos de al menos uno y típicamente dos, dominios de variable, en los cuales todos o sustancialmente todos los bucles hipervariables corresponden a aquellos de inmunoglobulina

no humana y todos o sustancialmente todos los FR son aquellos de una secuencia de inmunoglobulina humana, excepto para la o las sustituciones Fr como se anotaron anteriormente. El anticuerpo humanizado opcionalmente también comprenderá al menos una porción de una región constante de inmunoglobulina, típicamente aquella de la inmunoglobulina humana. Para detalles adicionales, ver 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).

El término "región hipervariable" cuando se utiliza aquí se refiere a residuos de aminoácido de un anticuerpo que son responsables por la unión de antígeno. La región hipervariable comprende residuos de aminoácido de una "región determinante de complementariedad" o "CDR" (por ejemplo los 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 eñl 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 "buque 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 "marco" o "FR" son aquellos residuos de dominio variable diferentes de los residuos de región hipervariable como se definió aquí.

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Un "anticuerpo desnudo" es un anticuerpo (como se define aquí) que no se conjuga a una molécula heteróloga, tal como una porción citotóxica o radiomarcada.

Ejemplos de anticuerpos que se unen al antígeno CD20 incluyen "C2B8", que es ahora denominado "Rituximab" ("RITUXAN ") (Patente US No. 5,736,137); el anticuerpo de murino 2B8 marcado con itrio-[90] designado "Y2B8" o "Ibritumomab Tiuxetan" (ZEVALIN®) comercialmente disponible de IDEC Pharmaceuticals, Inc. (Patente US No. 5,736,137; de 2B8 depositado con el ATCC bajo ascceso No. HB11388 en Junio 22, 1993); murino IgG2a "B1", también denominado "Tositumomab," opcionalmente marcado con ^{131}I para generar el "^{131}I-B1" o anticuerpo "yodo I131 tositumomab" (BEXXAR™) comercialmente disponible de Corixa (ver, también, Patente US No. 5,595,721); anticuerpo monoclonal de murino "1F5" (Press *et al. Blood* 69(2):584-591 (1987) y variantes de estos que incluyen "parche marco" o 1F5 humanizado (WO03/002607, Leung, S.; ATCC depósito HB-96450); anticuerpo 2H7 de murino y 2H7 quimérico (Patente US No. 5,677,180); 2H7 humanizado; huMax-CD20 (Genmab, Dinamarca, WO2004/035607); AME-133 (Applied Molecular Evolution); anticuerpo A20 o variantes de esto tales como el anticuerpo A20 quimérico o humanizado (cA20, hA20, respectivamente) o IMMU-106 (US 2003/0219433, Immunomedics); anticuerpos de unión CD20 que incluyen Leu-16 vaciado de epítopo, 1H4, o 2B8, opcionalmente conjugado con IL-2, como en la US 2005/0069545 A1 y WO 2005/16969 (Carr *et al*); anticuerpo biespecífico que se une a CD22 y CD20,

por ejemplo, hLL2xhA20 (WO2005/14618, Chang *et al*); los anticuerpos monoclonales L27, G28-2, 93-1B3, B-C1 o NU-B2 disponible de International Leukocyte Typing Workshop (Valentine *et al.* , En: *Leukocyte Typing* III (McMichael, Ed., p 440, Oxford University Press (1987)); 1H4 (Haisma *et al. Blood* 92:184 (1998)); conjugado auristatin E anti-CD20 (Seattle Genetics); anti-CD20-IL2 (EMD/Biovation/City of Hope); terapia anti-CD20 MAb (EpiCyte); y anticuerpo anti-CD20 TRU 015 (Trubion).

Los términos "Rituximab" o "RITUXAN®" se refieren aquí al anticuerpo monoclonal de murino/humano quimérico genéticamente trabajado con ingeniería dirigido contra el antígeno CD20 y designado "C2B8" en la Patente US No. 5,736,137, que incluye fragmentos de estos que retienen la capacidad de unirse a CD20. El Rituximab es comercialmente disponible de Genentech.

Puramente para los propósitos establecidos aquí y a menos que se indique otra cosa, "2H7 humanizado" se refiere a un anticuerpo humanizado que se une a CD20 humano, o un fragmento de unión de antígeno de este, en donde el anticuerpo es efectivo para vaciar células B de primate *in vivo*, el anticuerpo comprende la región variable de cadena H (V_H) de esta al menos una secuencia CDRH3 de la SEQ ID NO:12 (Figura 1B) de un anticuerpo CD20 antihumano y sustancialmente los residuos marco de consenso humano (FR) del subgrupo III de cadena pesada humana (V_HIII). En una modalidad preferida, este anticuerpo comprende además la secuencia CDR H1 de la cadena H de la SEQ ID NO: 10 y la

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secuencia CDR H2 de la SEQ ID NO: 11 , y más preferiblemente comprende además la secuencia CDR L1 de la cadena L de la SEQ ID NO:4, la secuencia CDR L2 de la SEQ ID NO:5, la secuencia CDR L3 de la SEQ ID NO:6 y sustancialmente los residuos marco de consenso humano(FR) del subgrupo I de cadena ligera humana (VI), en donde la región V_H se puede unir a una región constante de cadena IgG humana, en donde la región puede ser, por ejemplo, IgG1 o IgG3. En una modalidad preferida, tal anticuerpo comprende la secuencia V_H de la SEQ ID NO: 8 (v16, como se muestra en la Figura 1B), opcionalmente también comprende la secuencia V_L de la SEQ ID NO:2 (v16, como se muestra en la Figura 1A), la cual puede tener las sustituciones de aminoácido de la D56A y N100A en la cadena H y S92A en la cadena L (v96). Preferiblemente el anticuerpo es un anticuerpo intacto que comprende las secuencia de aminoácido de cadena ligera y pesada de la SEQ ID NOS: 13 y 14, respectivamente, como se muestra en las figuras 2 y 3. Otras modalidades preferidas es donde el anticuerpo es 2H7.v31 que comprende las secuencia de aminoácido de cadena pesada y ligera de la SEQ ID NOS:13 y 15, respectivamente, como se muestra en la Figuras. 2 y 4. El anticuerpo aquí puede además comprender al menos una sustitución de aminoácido en la región Fc que mejora la actividad ADCC y/o CDC, tal como una en donde las sustituciones de aminoácido son S298A/E333A/K334A, más preferiblemente 2H7.v31 que tiene la secuencia de aminoácido de cadena pesada de la SEQ ID NO: 15 (como se muestra en la Figura 4). Cualquiera de estos anticuerpos puede comprender además al menos una sustitución de aminoácido en la región Fc que disminuye la actividad CDC, por

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ejemplo, que comprende al menos la sustitución K322A. Ver Patente U.S. No. 6,528,624B1 (Idusogie *et al.*).

Un 2H7 humanizado preferido es un anticuerpo intacto o un fragmento de anticuerpo que comprende la secuencia de cadena ligera variable:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

y la secuencia de cadena pesada variable

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SS (SEQ ID NO:8).

Donde el anticuerpo 2H7 humanizado es un anticuerpo intacto, preferiblemente comprende la secuencia de aminoácido de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

y la secuencia de aminoácido de cadena pesada:

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EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

o la secuencia de aminoácido de cadena pesada:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

En la modalidad preferida de la invención, la región variable de las variantes basadas en la versión 16 2H7 tendrán la secuencia de aminoácido de v16 excepto en las posiciones de las sustituciones de aminoácido que están indicadas en la tabla de adelante. A menos que se indique otra cosa, las variantes 2H7 tendrán la misma cadena ligera que aquella de v16.

Versión 2H7	Cambios en cadena pesada (V_H)	Cambios en cadena ligera (V_L)	Cambios Fc
31	-	-	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N10	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L

Un anticuerpo "aislado" es aquel que ha sido identificado y separado y/o recuperado de un componente de su ambiente natural. Los componentes contaminantes de su ambiente natural son materiales que interferirían con el diagnóstico o los usos terapéuticos para el anticuerpo, y pueden incluir enzimas, hormonas, y otros solutos proteínicos o no proteínicos. En las modalidades preferidas, el anticuerpo se purificará (1) a más del 95% en peso del anticuerpo determinado por el método Lowry, y más preferiblemente más del 99% en peso, (2) a un grado suficiente para obtener al menos 15 residuos de la secuencia de aminoácido N-terminal o interna mediante el uso de un secuenciador de copa de giro, o (3) para homogeneidad por medio de SDS-PAGE bajo condiciones reductoras o no reductoras utilizando azul Coomassie o, preferiblemente, teñido de plata. El anticuerpo aislado incluye el anticuerpo in situ dentro de células recombinantes en razón a que al menos un componente del ambiente natural del anticuerpo no estará presente. Ordinariamente, sin embargo, el anticuerpo aislado se preparará mediante al menos una etapa de purificación.

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Un "sujeto" aquí es un sujeto humano. En general, el sujeto es elegible para tratamiento para esclerosis múltiple. Para los propósitos de esta, tal sujeto elegible es aquel que experimenta, ha experimentado, o probablemente experimente, uno o más signos, síntomas u otra indicación de esclerosis múltiple; se ha diagnosticado con esclerosis múltiple sea, por ejemplo, recientemente diagnosticada (con "nuevo inicio" de MS), previamente diagnosticada con una nueva recaída o exacerbación, previamente diagnosticada y en remisión, etc.; y/o este en riesgo de desarrollar esclerosis múltiple. Uno que sufra de o en riesgo de sufrir de esclerosis múltiple puede opcionalmente ser identificado como uno que se ha seleccionado por los niveles elevados de células B CD20 positivas en el suero, fluido cerebroespinal (CSF) y/o lesión o lesiones MS y/o se seleccione para utilizar un ensayo para detectar anticuerpos, evaluados cualitativamente, y preferiblemente cuantitativamente. Ejemplos de tales anticuerpos asociados con esclerosis múltiple incluyen proteína básica anti-mielina (MBP), glicoproteína oligodendrocítica anti-mielina (MOG), anticuerpos anti-ganglиósido y/o anti-neurofilamentos. Tales anticuerpos se pueden detectar en el suero del sujeto, en fluido cerebroespinal (CSF) y/o lesión MS. Por anticuerpo "elevado" o nivel o niveles de célula B se significan nivel o niveles de tales anticuerpos o células B que excedan significativamente el o los niveles de un individuo sin MS.

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"Tratamiento" de un sujeto se refiere aquí tanto a tratamiento terapéutico como profiláctico o medidas de prevención. Aquellos necesitados de tratamiento incluyen aquellos ya con esclerosis múltiple así como también aquellos en los cuales la esclerosis múltiple se va evitar. De esta manera, el sujeto que ha sido diagnosticado por tener esclerosis múltiple o puede estar predispuesto o susceptible a la esclerosis múltiple.

Un "síntoma" de MS es cualquier fenómeno mórbido o que se aparte de lo normal en la estructura, función, o sensación, experimentada por el sujeto e indicativo de MS.

"Esclerosis múltiple" se refiere a la enfermedad crónica y a menudo discapacitante del sistema nervioso central caracterizada por la destrucción progresiva de mielina. Existen cuatro formas internacionalmente reconocidas de MS, a saber, la esclerosis múltiple progresiva primaria (PPMS), la esclerosis múltiple recidivante-remitente (RRMS), la esclerosis múltiple progresiva secundaria (SPMS), y esclerosis múltiple progresiva recidivante(PRMS).

"La esclerosis múltiple progresiva primaria" o "PPMS" se caracteriza por una progresión gradual de la enfermedad proveniente de su inicio sin ninguna recaída y remisión superpuesta, pueden haber períodos de igualamiento de la actividad de la enfermedad y podrían haber buenos y malos días o semanas. La PPMS difiere de la RRMS y SPMS en que el inicio es

típicamente al final de los treinta o al comienzo de los cuarenta, con la misma probabilidad entre hombres y mujeres para desarrollarla, y la actividad de la enfermedad inicial es a menudo en la columna vertebral y no en el cerebro. La PPMS a menudo migra hacia el cerebro, pero es menos probable que dañe las áreas del cerebro que la RRMS o SPMS; por ejemplo las personas con PPMS tienen menos probabilidad de desarrollar problemas cognitivos. La PPMS es el subtipo de la MS que es menos probable que muestre lesiones inflamatorias (mejoramiento con gadolinio) o exploraciones MRI. La forma Progresiva Primaria de la enfermedad afecta entre el 10 y el 15% de todas las personas con esclerosis múltiple. La PPMS se puede definir de acuerdo con los criterios de McDonald *et al. Ann Neurol* 50:121-7 (2001). El sujeto con PPMS tratado aquí es usualmente uno con diagnóstico probable o definitivo de PPMS.

"Esclerosis múltiple recidivante-remitente" o "RRMS" se caracteriza por recaídas (también conocido como exacerbaciones) tiempo durante el cual pueden aparecer nuevos síntomas o resurgir viejos o empeorar. Los lapsos son seguidos por periodos de remisión, tiempo durante el cual la persona se recupera completa o parcialmente de lo déficit adquirido durante la recaída. Las recaídas pueden durar durante días, semanas o meses y la recuperación puede ser lenta y gradual o casi instantánea. La basta mayoría de las personas que presentan MS son primero diagnosticadas con RRMS. Esto es típicamente cuando ellas están en sus treinta o cuarenta, aunque diagnósticos más tempranos o

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tardíos son conocidos. Dos veces más mujeres que hombres presentan este subtipo de MS. Durante las recaídas, la mielina, un aislante protector cubre alrededor de las fibras nerviosas (neuronas) en las regiones de materia blanca del sistema nervioso central (CNS), se puede dañar en una respuesta inflamatoria al sistema inmune propio del cuerpo. Esto origina una amplia variedad de síntomas neurológicos que varía considerablemente dependiendo de cuales áreas del CNS este dañadas. Inmediatamente después de una recaída, la respuesta inflamatoria cesa y un tipo especial de célula glial en el CNS (denominado un oligodendrocito) patrocina la remielinización -un proceso mediante el cual se puede reparar la cubierta de mielina alrededor del axón. Es esta remielinización que puede ser responsable de la remisión. Aproximadamente 50% de los pacientes con RRMS la convierten a SPMS a los 10 años del inicio de la enfermedad. Después de los 30 años, esta figura se eleva al 90%. En cualquier momento, la forma recidivante-remitente de la enfermedad cuenta con alrededor del 55% de todas las personas como MS.

"La esclerosis múltiple progresiva secundaria o "SPMS" se caracteriza por una progresión constante del daño neurológico clínico con o sin recaídas superpuestas y remisiones y zonas estables menores. Las personas que desarrollan SPMS habrán experimentado previamente un periodo de RRMS que puede haber durado cualquiera desde dos a cuarenta años o más. Cualquier recaída superpuesta y remisiones que hallan, tienden a disminuir

gradualmente durante el tiempo. Desde el inicio de la fase progresiva secundaria de la enfermedad, la discapacidad inicia su avance mucho más rápidamente que lo que ocurrió durante la RRMS aunque el progreso puede ser aún muy lento en algunos individuos. Después de 10 años, el 50% de las personas con RRMS habrá desarrollado SPMS. A los 25 a 30 años, esa figura se habrá elevado al 90%. La SPMS tiende a estar asociados con niveles más bajos de formación de lesiones inflamatorias que en la RRMS pero la carga total de la enfermedad continua progresando. En cualquier momento, la SPMS cuenta con alrededor del 30% de todas las personas con esclerosis múltiple.

"Esclerosis múltiple progresiva recidivante" se refiere a "PRMS" se caracteriza por una progresión continua del daño neurológico clínico con recaídas y remisiones superpuestas. Existe una recuperación significativa inmediatamente luego de una recaída pero entre las recaídas existe un empeoramiento gradual de los síntomas. El PRMS afecta alrededor del 5% de todas las personas con esclerosis múltiple. Algunos neurólogos creen que el PRMS es una variante de la PPMS.

La expresión "cantidad efectiva" se refiere a una cantidad del anticuerpo (u otra droga) que es efectiva para prevenir, mejorar o tratar la esclerosis múltiple. Tal cantidad efectiva resultará en una mejora en los signos, síntomas u otras indicaciones de MS, tal como reducir la tasa de recaída, prevenir la discapacidad, reducir el número y/o volumen de lesiones MRI

del cerebro, mejorar el tiempo de caminado de 25 pies, extendiendo el tiempo a la progresión de la enfermedad (por ejemplo utilizando una escala de estado de discapacidad expandida, EDSS), etc.

"Exposición al anticuerpo" se refiere al contacto con o exposición al anticuerpo aquí en una o más dosis administradas durante un periodo de tiempo de aproximadamente 1-20 días. Las dosis pueden ser dadas una vez o en intervalos de tiempo fijo o irregulares durante este periodo de exposición. Las exposiciones a anticuerpo iniciales y tardías (por ejemplo segunda o tercera) son separada en el tiempo una de la otra como se describe en detalle aquí.

El término "agente inmunosupresivo" como se utiliza aquí para terapia adjunta se refiere a sustancias que actúan para suprimir o enmascarar el sistema inmune del mamífero que está siendo tratado aquí. Este incluiría sustancias que suprimen la producción de citoquina, subregulan o suprimen la expresión de autoantígeno, o enmascaran los antígenos MHC. Ejemplos de tales agentes incluyen pirimidina 2-amino-6-aril-5-sustituidas (ver Patente U.S. No. 4,665,077); drogas antiinflamatorias no esteroides (NSAID); ganciclovir, tacrolimus, glucocorticoides tales como Cortisol o aldosterona, agentes antiinflamatorios tales como un inhibidor de ciclooxigenasa, un inhibidor de 5-lipoxigenasa, o antagonista de receptor de leucotrieno; antagonistas de purina tales como azatioprina o mofetil

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micofenolato (MMF); agentes alquilantes tales como ciclofosfamida; bromocriptina; danazol; dapsona; glutaraldehído (que enascara los antígenos MHC, como se describieron en la Patente U.S. No. 4,120,649); anticuerpos antiidiotipos para antígenos MHC y fragmentos MHC; ciclosporina A; esteroides tales como corticosteroides o glucocorticosteroides o análogos de glucocorticoide, por ejemplo, prednisona, metilprednisolona, y dexametasona; inhibidores de dihidrofolato reductasa tales como metotrexato (oral o subcutáneo); hidroxicloroquina; sulfasalazina; leflunomida; citoquina o antagonistas de receptor de citoquina que incluyen anticuerpos anti-interferón-alfa, -beta, o -gama, anticuerpos alfa de factor de necrosis anti-tumoral (infliximab o adalimumab), inmunoahesina anti-TNF-alfa (etanercept), anticuerpos de factor-beta de necrosis anti-tumoral, anticuerpos anti-interleuquina-2 y anticuerpos del receptor IL-2; anticuerpos anti-LFA-1, que incluyen anticuerpos anti-CD11a y anti-CD18; anticuerpos anti-L3T4; globulina anti-linfocito heteróloga; anticuerpos pan-T, preferiblemente anticuerpos anti-CD3 o anti-CD4/CD4a; péptidos solubles que contienen un dominio de unión LFA-3 (WO 90/08187 publicado 7/26/90); estreptoquinasa; TGF-beta; estreptodornasa; ARN o ADN del huésped; FK506; RS-61443; desoxispergualina; rapamicina; receptor de célula T (Cohen *et al.*, Patente U.S. No. 5,114,721); fragmentos receptores de célula T (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294; Ianeway, *Nature*, 341: 482 (1989); y WO 91/01133); y anticuerpos receptores de célula T (EP 340,109) tales como T10B9.

El término "agente citotóxico" como se utiliza aquí se refiere a una sustancia que inhibe o previene la función de las células y/o causa la destrucción de las células. El término está destinado a incluir isótopos radiactivos (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 bacterias, hongos, plantas o de origen animal o fragmentos de estos.

Un "agente quimioterapéutico" es un compuesto químico en el tratamiento de cáncer. Ejemplos de agentes quimioterapéuticos incluyen agentes alquilatantes tales como tiotepa y CYTOXAN ciclosfosfamida; alquil sulfonatos tales como busulfan, improsulfan y piposulfan; aziridinas tales como benzodopa, carboquona, meturedopa, y uredopa; etileniminas y metilamelaminas que incluyen altretamina, trietilenomelamina, trietilenofosforamida, trietilenotiofosforamida y trimetilolmelamina; acetogeninas (especialmente bulatacina y bulatacinona); una camptotecina (que incluye el topotecan análogo sintético); briostatina; calistatina; CC- 1065 (que incluye su adozelesina, carzelesina y análogos sintéticos de bizelesina); criptoficinas (particularmente criptoficina 1 y criptoficina 8); dolastatina; duocarmicina (que incluye los análogos sintéticos, KW-2189 y CB1-TM1); eleuterobina; pancratistatina; una sarcodictina; espongistatina; mostazas de nitrógeno tales como clorambucil, clornafazina, colofosfamida, estramustina,

ifosfamida, mecloretamina, clorhidrato de óxido de mecloretamina, melfalan, novembiquina, fenesterina, prednimustina, trofosfamida, mostaza de uracilo; nitrosoureas tales como carmustina, clorozotocina, fotemustina, lomustina, nimustina, y ranimnustina; antibióticos tales como los antibióticos enediina (por ejemplo caliqueamicina, especialmente caliqueamicina gamaII y caliqueamicina omegaI1 (ver, por ejemplo, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dinemicina, incluyendo dinemicina A; bisfosfonatos, tales como clodronato; una esperamicina; así como también neocarzinostatina cromoforo y cromoforos antibióticos de cromoproteína enediina relacionada), aclacinomisinas, actinomicina, autramicina, azaserina, bleomicinas, cactinomicina, carabicina, carminomicina, carzinofilina, cromomicinis, dactinomicina, daunorubicina, detorubicina, 6-diazo-5-oxo-L-norleucina, ADRIAMYCIN® doxorubicina (que incluye morfolino-doxorubicina, cianomorfolino-doxorubicina, 2-pirrolino-doxorubicina y desoxidoxorubicina), epirubicina, esorubicina, idarubicina, marcelomicina, mitomicinas tales como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorubicina, estreptonigrina, estreptozocina, tubercidina, ubenimex, zinostatina, zorubicina; anti-metabolitos tales como metotrexato y 5-fluorouracilo (5-FU); análogos de ácido fólico tales como denopterina, metotrexato, pteropterina, trimetrexato; análogos de purina tales como fludarabina, 6-mercaptopurina, tiamiprina, tioguanina; análogos de pirimidina tales como ancitabina, azacitidina, 6-azauridina, carmofur, citarabina, dideoxiuridina,

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doxifluridina, enocitabina, floxuridina; andrógenos tales como calusterona, dromostanolona propionato, epitiostanol, mepitiostano, testolactona; anti-adrenales tales como aminoglutetimida, mitotano, trilostana; recargador de ácido fólico tales como ácido frolínico; aceglatona; aldofosfamida glicosida; ácido aminolevulínico; eniluracilo; amsacrina; bestrabucil; bisantreno; edatraxato; defofamina; demecolcina; diaziquona; elfornitina; acetato de eliptinio; una epotilona; etoglucida; nitrato de galio; hidroxiurea; lentinan; lonidainina; maitansinoidas tales como maitansina y ansamitocinas; mitoguazona; mitoxantrona; mopidanmol; nitraerina; pentostatina; fenamet; pirarubicina; losoxantrona; ácido podofilínico; 2-etilhidrazida; procarbazina; complejo polisacárido PSK® (JHS Natural Products, Eugene, OR); razoxano; rizoxina; esizofiran; espirogermanio; ácido tenuazónico; triaziquona; 2,2',2''-triclorotrietilamina; tricotecenos (especialmente toxina T-2, verracurin A, roridina A y anguidina); uretano; vindesina; dacarbazina; manomustina; mitobronitol; mitolactol; pipobroman; gacitosina; arabinosida ("Ara-C"); ciclofosfamida; tiotepa; taxoidas, por ejemplo, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, NJ.), ABRAXANE™ Cremofor-libre, formulación de nanopartícula construida por ingeniería-albúmina de paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), y TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); cloranbucil; GEMZAR® gemcitabina; 6-tioguanina; mercaptopurina; metotrexato; análogos de platino tales como cisplatina y carboplatina; vinblastina; platino; etoposida (VP-16);

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ifosfamida; mitoxantrona; vincristina; NAVELBINE® vinorelbina; novantrona; teniposida; edatrexato; daunomicina; aminopterina; xeloda; ibandronato; CPT-11; inhibidor de topoisomerasa RFS 2000; difluorometilornitina (DMFO); retinoides tales como ácido retinoico; capecitabina; y sales farmacéuticamente aceptables, ácidos o derivados de cualquiera de los anteriores.

También incluidos en esta definición están los agentes antihormonales que actúan para regular o inhibir la acción de la hormona sobre los tumores tales como anti-estrógenos y moduladores del receptor de estrógeno selectivo (SERMs), que incluye, por ejemplo, tamoxifeno (que incluye NOLVADEX® tamoxifeno), raloxifeno, droloxifeno, 4-hidroxitamoxifeno, trioxifeno, keoxifeno, LY117018, onapristona, y FARESTON toremifeno; inhibidores de aromatasa que inhiben la enzima aromatasa, que regulan la producción de estrógeno en glándulas adrenales, tales como, por ejemplo, 4(5)-imidazoles, aminoglutetimida, MEGASA® megestrol acetato, AROMASIN exemestano, formestanie, fadrozol, RTVTSOR® vorozol, FEMARA® letrozol, y ARJMIDEX® anastrozol; y anti-andrógenos tales como flutamida, nilutamida, bicalutamida, leuprolida, y goserelina; así como también troxacitabina (un análogo de 1,3-dioxolano nucleósido citosina); oligonucleótidos antisentido, particularmente aquellos que inhiben la expresión de genes en la señalización de células implicadas en la proliferación de célula aberante, tales como, por ejemplo, PKC-alfa, Ralf y H-Ras; vacunas tales como vacunas de terapia génica, por ejemplo, la

vacuna ALLOVECTINA®, vacuna LEUVECTINA®, y la vacuna VAXID®; PROLEUKIN® rIL-2; LURTOTECAN® inhibidor de topoisomerasa 1; ABARELIX® rmRH; y sales farmacéuticamente aceptables, ácidos derivados de de cualquiera de los anteriores.

El término "citoquina" es un término genérico para proteínas liberadas por una población de células que actúan sobre otra célula como mediadores intercelulares. Ejemplos de tales citoquinas son linfoquinas, monoquinas; interleuquinas (IL) tales como IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL- 15; un factor de necrosis tumoral tal como TNF-α o TNF-β; y otros factores de polipéptido que incluyen el LIF y un ligando de kit (KL). Como se utiliza aquí, el término citoquina incluye proteínas de fuentes naturales o de cultivo de célula recombinante y equivalentes biológicamente activos de citoquinas de secuencia nativa, que incluyen entidades de molécula pequeña sintéticamente producidas y derivados farmacéuticamente aceptables y sales de estos.

El término "hormona" se refiere a hormonas de polipéptido, que son generalmente secretadas mediante órganos glandulares con ductos. Incluidas entre las hormonas están, por ejemplo, la hormona del crecimiento tal como la hormona del crecimiento humano, la hormona del crecimiento humano N-metionilo, y la hormona de crecimiento bovino; hormona paratiroide; tiroxina; insulina; proinsulina; relaxina; prorelaxina; hormonas de glicoproteína tales como la hormona estimulante del folículo

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(FSH), hormona estimulante tiroide (TSH), y hormona luteinizante (LH); prolactina, lactógeno de placenta, péptido asociado con gonadotropina de ratón, inhibina; activina; sustancia inhibidora de muleriana; y trombopoyetina. Como se utiliza aquí, el término hormona incluye proteínas provenientes de fuentes naturales o de cultivo de célula recombinante y equivalentes biológicamente activos de la hormona de secuencia nativa, que incluye entidades de molécula pequeña sintéticamente producida y derivados farmacéuticamente aceptables y sales de estos.

El término "factor de crecimiento" se refiere a proteínas que promueven el crecimiento, e incluyen, por ejemplo, factor de crecimiento hepático; factor de crecimiento de fibroblasto; factor de crecimiento endotelial vascular; factores de crecimiento nervioso tal como NGF-β; factor de crecimiento derivado de plaqueta; factores de crecimiento transformantes (TGF) tales como TGF-α y TGF-β; factor I y -Ii de crecimiento similar a insulina; eritropoyetina (EPO); factores osteoinductivos; interferonas tales como interferona-α, -β, y -γ; y factores estimulantes de colonia (CSFs) tales como macrófago-CSF (M-CSF); granulocito-macrófago-CSF (GM-CSF); y granulocito-CSF (G- CSF). Como se utiliza aquí, el término factor de crecimiento incluye proteínas provenientes de fuentes naturales o de cultivo de célula recombinante y equivalentes biológicamente activos de factor de crecimiento de secuencia nativa, que incluyen entidades de molécula pequeña sintéticamente producidas y derivados farmacéuticamente aceptables y sales de estos.

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El término "integrina" se refiere a una proteína receptora que le permite a las células tanto unirse como responder a la matriz extracelular y está involucrada en una variedad de funciones celulares tales como el curación de heridas, diferenciación celular, ubicación de células tumorales y apoptosis. Ellas son parte de una gran familia de receptores de adhesión de célula que están involucrados en la matriz extracelular de la célula y en interacciones célula-célula. Las integrinas funcionales consisten de dos subunidades de glicoproteína de transmembrana, denominadas alfa y beta, que no están covalentemente unidas. Las subunidades alfa comparten todas alguna homología una con la otra, como lo hacen las subunidades beta. Los receptores siempre contienen una cadena alfa y una cadena beta. Ejemplos incluyen Alfa6beta1, Alfa3beta1, alfa7beta1, LFA-1, alfa 4, integrina etc. Como se utiliza aquí, el término integrina incluye proteína de fuentes naturales o de cultivo de célula recombinante y equivalentes biológicamente activos de la integrina de secuencia nativa, que incluye entidades de molécula pequeña sintéticamente producida y derivados farmacéuticamente aceptables y sales de estos.

Ejemplos de "antagonistas de anticuerpos de integrina" incluyen aquí un anticuerpo LFA-1 tal como Efalizumab (RAPTIV A®) comercialmente disponible de Genentech; un anticuerpo de integrina alfa 4 tal como natalizumab (TYSABRI®) disponible de Biogen; derivados de fenilalanina diazacíclica (WO 2003/89410);

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derivados de fenilalanina (WO 2003/70709, WO 2002/26830, WO 2002/16329 y WO 2003/53926); derivados de ácido fenilpropionico (WO 2003/10135); derivados de enamina (WO 2001 /79173); derivados de ácido propanóico (WO 2000/37444); derivados de ácido alcanóico (WO 2000/32575); derivados de fenilo sustituido (Patentes US Nos. 6,677,339 y 6,348,463); derivados de amina aromático (Patente US No. 6,369,229); y polipéptido de dominio de disintegrina ADAM (US2002/0042368), anticuerpos a la integrina alfavbeta3 (EP 633945); derivados de aminoácido biciclo aza-puenteado (WO 2002/02556) etc.

Para los propósitos de esto, "el factor alfa de necrosis tumoral (TNF-alfa)" se refiere a una molécula de TNF-alfa humana que comprende la secuencia de aminoácido como se describe en Pennica *et al.*, *Nature*, 312:721 (1984) o Aggarwal *et al*, *JBC*, 260:2345 (1985).

Un "inhibidor de TNF-alfa" aquí es un agente que inhibe, en alguna proporción una función biológica de TNF-alfa, generalmente a través de la unión a TNF-alfa y neutralizando su actividad. Ejemplos de inhibidores TNF específicamente contemplados aquí son Etanercept (ENBREL®), Infliximab (REMICADE®) y Adalimumab (HUMIRA™).

Ejemplos de "fármacos antirreumáticos con capacidad de modificación de la enfermedad" "DMARD" incluyen hidroxicloroquina, sulfasalazina, metotrexato, leflunomida,

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etanercept, infliximab (más metrotrexato oral y subcutáneo), azatioprina, D-penicilamina, Gold (oral), Gold (intramuscular), minociclina, ciclosporina, inmunoabsorción de proteína A de estafilococo, que incluye sales y derivados de estos, etc.

"Ejemplos de drogas antiinflamatorias no esteroides" "NSAID" son ácido acetisalicílico, ibuprofeno, naproxeno, indometacina, sulindac, tolmetina, que incluye sales y derivados de estos, etc.

"Corticosteroide" se refiere a una cualquiera de varias sustancias de ocurrencia natural o sintética con la estructura química de los esteroides que semejan o aumentan los efectos de los corticosteroides de ocurrencia natural. Ejemplos de corticosteroides sintéticos incluyen prednisona, prednisolona (incluyendo metilprednisolona), dexametasona, glucocorticoide y betametasona.

Un "inserto de paquete" se utiliza para referirse a instrucciones de costumbre incluidas en paquetes comerciales o en productos terapéuticos, que contienen la información acerca de la indicación, uso, dosis, administración, contraindicaciones, otros productos terapéuticos para ser combinados con el producto empacado, y/o prevenciones con relación al uso de tales productos terapéuticos, etc.

II. Terapia

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La presente invención suministra un método para tratar esclerosis múltiple en un sujeto que sufre de esta, que comprende administrar una cantidad efectiva de un anticuerpo que se une al marcador de superficie de célula B (preferiblemente un anticuerpo CD20) al sujeto. La MS al ser tratada aquí incluye esclerosis múltiple progresiva primaria (PPMS), esclerosis múltiple recidivante-remitente (RRMS), esclerosis múltiple progresiva secundaria (SPMS), y esclerosis múltiple progresiva recidivante (PRMS), pero la terapia de cualquiera la PPMS o RRMS son las modalidades preferidas aquí.

De acuerdo a un protocolo de dosificación preferido, el método comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto MS para suministrar una exposición al anticuerpo inicial de aproximadamente 5 a 4 gramos (preferiblemente aproximadamente 1.5 a 2.5 gramos) seguido por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos (preferiblemente aproximadamente 1.5 a 2.5 gramos), la segunda exposición a anticuerpo no siendo suministrada hasta desde aproximadamente 16 a 60 semanas desde la exposición inicial del anticuerpo. Para los propósitos de esta invención, la segunda exposición de anticuerpo es la siguiente vez que el sujeto es tratado con anticuerpo CD20 después de la exposición inicial al anticuerpo, no habiendo intervención de tratamiento de anticuerpo CD20 o exposición entre las exposiciones inicial y segunda.

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El intervalo entre las exposiciones de anticuerpo inicial o segunda o subsecuente se puede medir de cualquiera la primera o la segunda dosis de la exposición inicial al anticuerpo, pero preferiblemente de la primer dosis de la exposición inicial al anticuerpo.

En las modalidades preferidas aquí, las exposiciones al anticuerpo son aproximadamente de 24 semanas o 6 meses de diferencia; o aproximadamente 48 semanas o 12 meses de diferencia.

En una modalidad, la segunda exposición al anticuerpo no se suministra hasta aproximadamente 20 a 30 semanas desde la exposición inicial, opcionalmente seguida por una tercera exposición de anticuerpo de aproximadamente 0.5 a 4 gramos (preferiblemente aproximadamente 1.5 a 2.5 gramos), la tercer exposición no siendo administrada hasta desde aproximadamente 46 a 60 semanas (preferiblemente desde aproximadamente 46 a 54 semanas) desde la exposición inicial, y luego, preferiblemente ninguna exposición a anticuerpo adicional se suministra hasta al menos aproximadamente 70-75 semanas desde la exposición inicial.

En una modalidad alternativa, no se suministra segunda exposición al anticuerpo hasta aproximadamente 46 a 60 semanas desde la exposición inicial, y las subsecuentes exposiciones a anticuerpo, si existe alguna, no se suministran hasta

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aproximadamente 45 a 60 semanas desde la exposición previa al anticuerpo.

Una cualquiera o más de las exposiciones al anticuerpo aquí se pueden suministrar al sujeto como una dosis simple de anticuerpo, o como dos dosis separadas del anticuerpo (es decir que constituyen una primera y segunda dosis). El número particular de dosis (sea una o dos) empleada para cada exposición a anticuerpo es dependiente, por ejemplo, del tipo de MS tratado, el tipo de anticuerpo empleado, y que tipo de segundos medicamentos se emplea, y el método y frecuencia de administración. Cuando se administran dos dosis separadas, la segunda dosis es preferiblemente administrada desde aproximadamente 3 a 17 días, más preferiblemente desde aproximadamente 6 a 16 días, más preferiblemente desde 13 a 16 días desde el momento de que la primer dosis fue administrada. Cuando se administran dos dosis separadas, la primera y segunda dosis del anticuerpo es preferiblemente aproximadamente 0.5 a 1.5 gramos, más preferiblemente aproximadamente 0.75 a 1.3 gramos.

En una modalidad, el sujeto suministra al menos aproximadamente tres, o al menos cuatro exposiciones del anticuerpo, por ejemplo, desde aproximadamente 3 a 60 exposiciones, y más particularmente aproximadamente 3 a 40 exposiciones, más particularmente, aproximadamente 3 a 20 exposiciones. Preferiblemente, tales exposiciones son administradas a intervalos cada una de 24 semanas a 6 meses, o 48

semanas a 12 meses. En una modalidad cada exposición a anticuerpo se suministra en una dosis simple del anticuerpo. En una modalidad alternativa, cada exposición al anticuerpo se suministra como dos dosis separadas del anticuerpo. Sin embargo, no cada exposición al anticuerpo necesita ser suministrada en una dosis simple o como dos dosis separadas.

El anticuerpo puede ser un anticuerpo desnudo o puede ser conjugado con otras moléculas tales como agente citotóxico como un compuesto radioactivo. El anticuerpo preferido aquí es Rituximab, 2H7 humanizado (por ejemplo que comprende las secuencias de dominio variable en la SEQ ID NOS. 2 y 8) o 2H7 humanizado que comprende secuencias de dominio variable en la SEQ ID NOS. 23 y 24, o huMax-CD20 (Genmab).

En una modalidad, el sujeto nunca ha sido tratado previamente con droga o drogas, tales como agente o agentes inmunosupresores, para tratar la esclerosis múltiple y/o nunca ha sido tratado previamente con un anticuerpo ha un marcador de superficie de célula B (por ejemplo nunca previamente tratado con anticuerpo CD20).

El anticuerpo se administra por cualesquier medios adecuados, incluyendo parenteral, tópica, subcutáneo, intraperitoneal, intrapulmonar, intranasal, y/o administración intralesional. Las infusiones parenterales incluyen administración intramuscular, intravenosa, intraarterial,

intraperitoneal, o subcutánea. La administración intratecal también se contempla (ver, por ejemplo, solicitud de Patente US No. 2002/0009444, Grillo-Lopez, un suministro intratecal concerniente de un anticuerpo CD20). Además, el anticuerpo puede ser adecuadamente administrado mediante infusión de pulso, por ejemplo, con dosis declinantes del anticuerpo. Preferiblemente, la dosificación es dada intravenosamente, subcutáneamente o intratecalmente, más preferiblemente mediante infusión o infusiones intravenosas.

Aunque el anticuerpo CD20 puede ser solamente administrado con droga al sujeto para tratar la esclerosis múltiple uno podría opcionalmente administrar un segundo medicamento, tal como un agente citotóxico, agente quimioterapéutico, agente inmunosupresor, citoquina, antagonista de citoquina o anticuerpo, factor de crecimiento, hormona, integrina, antagonista de integrina o anticuerpo (por ejemplo un anticuerpo LFA-1 tal como efalizumab (RAPTIVA®) comercialmente disponible de Genentech, o anticuerpo de integrina alfa 4 tal como natalizumab (TYSABRI) disponibles de Biogen) etc., con el anticuerpo que se une al marcador de superficie de la célula B (por ejemplo con el anticuerpo CD20).

En la modalidad preferida de terapia de combinación, el anticuerpo se combina con una droga de la clase de interferón tal como IFN-beta-1a (REBIF® y AVONEX®) o IFN-beta-1b (BETASERON); un oligopéptido tal como glatiramer acetato (COPAXONE®); un

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agente citotóxico tal como mitoxantrona (NOVANTRONA®), metotrexato, ciclofosfamida, clorambucil, azatioprina; inmunoglobulina intravenosa (gama inmunoglobulina); terapia de vaciamiento de linfocito (por ejemplo mitoxantrona, ciclofosfamida, Campath, anti-CD4, cladribina, irradiación del cuerpo total, trasplante de médula ósea); corticosteroide (por ejemplo metilprednisolona, prednisona, dexametasona, o glucorticoides), que incluye terapia de corticosteroide sistémica; terapia inmunosupresora de vaciamiento no linfocito (por ejemplo, mofetil micofenolato (MMF) o ciclosporina); droga que disminuye el colesterol de la clase "estatina", que incluye cerivastatina (BAYCOL®), fluvastatina (LESCOL®), atorvastatina (LIPITOR®), lovastatina (MEVACOR®), pravastatina (PRAV ACHOL®), Simvastatina (ZOCOR®); estradiol; testosterona (opcionalmente a dosis elevadas; Stuve *et al. Neurology* 8:290-301 (2002)); terapia de reemplazo de hormona; tratamiento para síntomas secundarios relacionados a MS (por ejemplo espasticidad, incontinencia, dolor, fatiga); un inhibidor de TNF; una droga antirreumática con capacidad de modificación de la enfermedad (DMARD); droga antiinflamatoria no esteroide (NSAID); plasmaféresis; levotiroxina; ciclosporina A; análogo de somatastatina; citoquina o antagonista receptor de citoquina; anti-metabolito; agente inmunosupresor; cirugía de rehabilitación; radioyodo; tiroidectomía; otro antagonista/anticuerpo de superficie de célula B; etc.

El segundo medicamento se administra con la exposición inicial y/o exposiciones finales del anticuerpo CD20, tal como la administración combinada incluye la coadministración, utilizando formulaciones separadas o formulaciones farmacéuticas simples y la administración consecutiva en cualquier orden, en donde preferiblemente existe un periodo aunque ambos (o todos) los ingredientes activos ejerzan simultáneamente sus actividades biológicas.

Además de la administración de los anticuerpos al sujeto, la presente solicitud contempla la administración de anticuerpos mediante terapia génica. Tal administración de ácido nucleico que codifica al anticuerpo está comprendida mediante la expresión administrar una "cantidad efectiva" de un anticuerpo, ver, por ejemplo, WO96/07321 publicado en marzo 14, 1996 que se relaciona con el uso de terapia génica para generar anticuerpos intracelulares.

Existen dos aproximaciones principales para conseguir el ácido nucleico (opcionalmente contenido en un vector) en las células de los sujetos; *in vivo* y *ex vivo*. Para suministro *in vivo* del ácido nucleico es inyectado directamente en el sujeto, usualmente en el sitio donde se requiere el anticuerpo. Para tratamiento *ex vivo*, las células del sujeto son removidas, el ácido nucleico es introducido en las células aisladas y las células modificadas son administradas al sujeto directamente o, por ejemplo, encapsuladas en membranas porosas que son

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implantadas en el sujeto (ver, por ejemplo, Patentes U.S. Nos. 4,892,538 y 5,283,187). Existe una variedad de técnicas disponibles para introducir ácidos nucleicos en células viables. Las técnicas varían dependiendo de si el ácido nucleico es transferido a células cultivadas *in vitro*, o *in vivo* en las células del huésped pretendido. Las técnicas adecuadas para la transferencia de ácido nucleico a células de mamífero *in vitro* incluyen el uso de liposomas, electroporación, microinyección, fusión de célula, DEAE-dextrano, el método de precipitación de fosfato de calcio, etc. Un vector comúnmente para suministro *ex vivo* del gen es un retrovirus.

Las técnicas de transferencia de ácido nucleico *in vivo* habitualmente preferidas incluyen la transfección con vectores virales (tal como adenovirus, virus Herpes simple I o virus adenoasociados) y sistemas basados en lípidos (lípidos útiles para transferencia mediada por lípido u otros genes DOTMA, DOPE y DC-Chol, por ejemplo). En algunas situaciones es deseable suministrar la fuente de ácido nucleico con un agente que apunta a las células objetivo tal como un anticuerpo específico para una proteína de membrana de superficie celular o la célula objetivo, un ligando para un receptor sobre la célula objetivo, etc. cuando se emplean liposomas, las proteínas que se unen a la proteína de membrana de superficie celular asociadas con endocitosis se puede utilizar para apuntar y/o facilitar la toma, por ejemplo, proteínas cápsido o fragmentos trópicos de estos para un tipo de célula particular, anticuerpos para proteína que sufren

internalización en ciclamiento, y proteínas que apuntan la localización intracelular. y la mejoran la vida media intracelular. La técnica de endocitosis mediada por receptor se describe, por ejemplo, por Wu *et al., J. Biol. Chem.* 262:4429-4432 (1987); y Wagner *et al., Proc. Natl. Acad. Sci USA* 87:3410-3414 (1990). Para revisión de los protocolos de marcación de en y terapia de en conocidos habitualmente ver Anderson *et al., Science* 256:808-813 (1992). Ver también la WO 93/25673 y las referencias citadas aquí.

III. Producción de Anticuerpos

Los métodos y artículos de elaboración de la presente invención usan, o incorporan un anticuerpo que se une al marcador de superficie de célula B, especialmente uno que se une a CD2. de acuerdo con esto, los métodos para generar tales anticuerpos se describirán aquí.

El marcador de superficie de célula B a ser utilizado para la producción de, o selección de, anticuerpos puede ser, por ejemplo, una forma soluble del marcador, o una porción de esta, que contiene un epítopo deseado. Alternativamente, o adicionalmente las células que expresan el marcador en su superficie celular se pueden utilizar para generar, o seleccionar, anticuerpos. Otras formas de marcador de superficie de célula B útiles para generar anticuerpos serán evidentes para aquellos expertos en la técnica.

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Una descripción sigue a las técnicas de ejemplo para la producción de los anticuerpos utilizados de acuerdo con la presente invención.

(i) Anticuerpos policlonales

Los anticuerpos policlonales son preferiblemente generados en animales mediante inyecciones subcutáneas (sc) o intraperitoneales (ip) múltiples de antígeno relevante y un adyuvante. Esta puede ser útil para conjugar el antígeno relevante a una proteína que es inmunogénica en las especies a ser inmunizadas, ore ejemplo, hemocianina de lapa ojo de cerradura, suero albúmina, tiroglobulina bovina, o inhibidor de tripsina de fríjol de soya que utiliza un agente bifucional o derivatizante, por ejemplo, maleimidobenzoil sulfosuccinimida éster (conjugación a través de residuos de cisteína), N-hidroxisuccinimida (a través residuos de lisina), glutaraldehído, anhídrido succínico, $SOCl_2$, o $R^1N=C=NR$, en donde R y R^1 son diferentes grupos de alquilo.

Los animales son inmunizados contra el antígeno, conjugados inmunogénicos, o derivados al combinar, por ejemplo, 100 µg o 5 µg de la proteína o conjugado (para conejos o ratones, respectivamente) con 3 volúmenes de adyuvante completo de Freund e inyectar la solución intradérmicamente en múltiples sitios. Un mes más tarde los animales son reforzados con 1/5 a 1/10 de la

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cantidad original del péptido o conjugado en un adyuvante completo de Freund mediante inyección subcutánea en sitios múltiples. 7 a 14 días más tarde los animales son sangrados y el suero es ensayado para título de anticuerpo. Los animales son esforzados hasta que el título se estabiliza. Preferiblemente, el animal es reforzado con el conjugado del mismo antígeno, pero conjugado a un proteína diferente y/o a través de un agente reticulante diferente. Los conjugados también se pueden hacer en cultivos de célula recombinante, como fusiones de proteína. También, los agentes agregantes tales como alumbre son adecuadamente utilizados para mejorar la respuesta inmune.

(ii) Anticuerpos monoclonales

Los anticuerpos monoclonales son obtenidos 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 para posibles variantes que surgen durante la producción fórmula anticuerpo monoclonal, tales variantes generalmente están presentes en cantidades menores. Así, el modificador "monoclonal" indica el carácter del anticuerpo como no siendo una mezcla de anticuerpos discretos o policlonales.

Por ejemplo, los anticuerpos monoclonales pueden hacerse utilizando el método de hibridoma primero descrito por Kohler et

al., Nature, 256:495 (1975), se puede hacer mediante métodos de ADN recombinante (Patente U.S. No. 4,816,567).

En el método de hibridoma, un ratón u otro animal huésped apropiado, tal como un hámster, se inmuniza como se describió anteriormente para elicitar linfocitos que producen o son capaces de producir anticuerpos que se unirán específicamente a la proteína utilizada para inmunización. Alternativamente, los linfocitos pueden ser inmunizados *in vitro*. Los linfocitos son entonces fusionados con células de mieloma utilizando un agente de fusión adecuado, tal como polietilenglicol, para formar una célula de hibridoma (Goding, *Monoclonal Antibodies: Principles y Practice*, pp.59-103 (Academic Press, 1986)).

Las células de hibridoma preparadas así son sembradas y se hacen crecer en un medio de cultivo adecuado que contienen preferiblemente una o más sustancias que inhiben el crecimiento o supervivencia de células de mieloma parentales no infundidas. Por ejemplo, si a las células de mieloma parental les falta la enzima hipoxantina guanina fosforibosil transferasa (HGPRT o HPRT), el medio de cultivo para los hibridomas típicamente incluirá hipoxantina, aminopterina, y timidina (medio HAT), cuyas sustancias evitan el crecimiento de las células deficientes en HGPRT.

Las células de mieloma preferidas son aquellas que se fusionan eficientemente, soportan producción de nivel alto

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estable de anticuerpo mediante células de producción de anticuerpo seleccionadas, y son sensibles a un medio tal medio HAT. Entre estos, las líneas de célula de mieloma preferida son líneas de mieloma de murino, tal como aquellas 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 o X63-Ag8-653 disponibles del American Type Culture Collection, Rockville, Mariland USA. Las líneas de célula de heteromieloma de mieloma humano y de mieloma de ratón también ha sido descritas para la producción de anticuerpos monoclonales (Kozbor, *J. Immunol*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

El medio de cultivo en el cual las células de hibridoma crecen se ensaya para producción de anticuerpos monoclonales dirigidos contra el antígeno. Preferiblemente, la especificidad de unión de los anticuerpos monoclonales producidos por las células de hibridoma se determina mediante la inmunoprecipitación o mediante un ensayo de unión *in vitro*, tal como radioinmunoensayo (RIA) o ensayo inmunoabsorbente ligado a enzima (ELISA).

La afinidad de unión del anticuerpo monoclonal puede, por ejemplo, ser determinada por el análisis Scatchard de Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

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Después de que se identifican las células de hibridoma que producen anticuerpos de la especificidad deseada, la afinidad, y/o actividad, los clones se pueden subclonar al limitar los procedimientos de dilución y ser crecidos mediante métodos estándar (Goding, *Monoclonal Antibodies: Principles and Practice,* pp.59-103 (Academic Press, 1986)). El medio de cultivo adecuado para este propósito incluye, por ejemplo, medio D-MEM o RPMI-1640. Además las células de hibridoma se pueden hacer crecer *in vivo* como tumores ascites en un animal.

Los anticuerpos monoclonales secretados mediante subclones son adecuadamente separados de medio de cultivo, fluido de ascites o suero mediante procedimientos de purificación de inmunoglobulina convencionales tales como, por ejemplo, proteína A-sefarosa, cromatografía de hidroxilapatita, electroforesis de gel, diálisis, o cromatografía de afinidad.

El ADN que codifica los anticuerpos monoclonales se aísla fácilmente y se secuencia utilizando procedimientos convencionales (por ejemplo al utilizar sondas de oligonucleótido que son capaces de unirse específicamente a genes que codifican las cadenas pesada y ligera de anticuerpos de murino). Las células de hibridoma sirven como una fuente preferida de tal ADN. Una vez aislado, el ADN se puede ubicar en vectores de expresión que entonces transfectados en células huéspedes tales como células de *E. coli*, células COS de simio, células de ovario de hámster chino (CHO), o células de mieloma que no producen de otra

manera proteína de inmunoglobulina, para obtener la síntesis de anticuerpos monoclonales en las células huésped recombinantes. Los artículos de revisión 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 modalidad adicional, los anticuerpos o fragmentos de anticuerpos se pueden aislar de librerías de fago de anticuerpos generadas utilizando 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) describe el aislamiento de anticuerpos de murino y humano, respectivamente, utilizando librerías de fago. Las publicaciones subsecuentes describen la producción de anticuerpos humanos de alta afinidad (rango nM) mediante arrastrado de cadena (Marks *et al., Bio/Technology,* 10:779-783 (1992)), así como también como infección combinatoria en recombinación *in vivo* como una estrategia para construir librerías de fago muy grandes (Waterhouse *et al., Nuc. Acids. Res.,* 21:2265-2266 (1993)). Así, estas técnicas son alternativas viables a las técnicas de hibridoma de anticuerpo monoclonal para el aislamiento de anticuerpos monoclonales.

El ADN también se puede modificar, por ejemplo, al sustituir las secuencia de codificación para dominios constantes de cadena pesada y ligera en lugar de secuencias de murino homólogas

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(Patente U.S. No. 4,816,567; Morrison, *et al., Proc. Natl Acad. Sci USA,* 81:6851 (1984)), o unirse covalentemente a la secuencia codificante de inmunoglobulina toda o parte de la secuencia codificante de un polipéptido de no-inmunoglobulina.

Típicamente tales polipéptidos de no-inmunoglobulina son sustituidos por los dominios constantes de un anticuerpo, o ellos son sustituidos por los dominios variables de un sitio que combina antígeno de un anticuerpo para crear un anticuerpo bivalente quimérico que comprende un sitio que combinan antígeno que tiene especificidad para un antígeno y otro sitio que combina otro antígeno que tiene especificidad para un antígeno diferente.

(iii) Anticuerpos humanizados

Los métodos para los anticuerpos no humanos humanizantes se han descrito en la técnica. Preferiblemente, un anticuerpo humanizado tiene uno o más residuos de aminoácido introducidos en este desde una fuente que es no humana. Estos residuos de aminoácido no humanos san a menudo de nominados como residuos de "importación" que son típicamente tomados desde un dominio variable de "importación". La humanización puede ser esencialmente desarrollada siguiendo el método de Winter y co-trabajadores (Jones *et al., Nature,* 321:522-525 (1986); Riechmann *et al., Nature,* 332:323-327 (1988); Verhoeyen *et al., Science,* 239:1534-1536 (1988)), al sustituir secuencias de región hipervariable para las secuencias correspondientes del anticuerpo

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humano. De acuerdo con esto, tales anticuerpos "humanizados" son anticuerpos quiméricos (Patente U.S. No. 4,816,567) en donde sustancialmente menos que un dominio variable humano intacto ha sido sustituido por la secuencia correspondiente de las especies no humanas. En la práctica, los anticuerpos humanizados son típicamente anticuerpos humanos en los cuales algunos residuos de región hipervariable y posiblemente algunos residuos FR son sustituidos por residuos de sitios análogos en anticuerpos de roedor.

La elección de los dominios variables humanos, tanto ligeros como pesados, a ser utilizados en la elaboración de anticuerpos humanizados es muy importante para reducir la antigenicidad. De acuerdo con esto el método de "mejor ajuste" así llamado, la secuencia del dominio variable de un anticuerpo de roedor es seleccionada contra la librería completa de secuencias de dominio variable humanas conocidas. La secuencia humana que es más cercana a aquella del roedor es luego aceptada como la región marco humana (FR) para el anticuerpo humanizado (Sims *et al., J. Immunol,* 151:2296 (1993); Chothia *et al., J. Mol Biol,* 196:901 (1987)). Otro método utiliza una región marco particular derivada de una secuencia de consenso de todos los anticuerpos humanos de un subgrupo particular de regiones variables de cadena ligera y pesada. El mismo marco se puede utilizar para varios diferentes anticuerpos humanizados (Carter *et al., Proc. Natl Acad. Sci. USA,* 89:4285 (1992); Presta *et al., J. Immunol,* 151: 2623 (1993)).

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Es además importante que los anticuerpos sean humanizados con retención de alta afinidad de antígeno y otras propiedades biológicas favorables. Para lograr esta meta, de acuerdo a un método preferido, los anticuerpos humanizados son preparados mediante un proceso de análisis de las secuencias parentales y varios productos conceptuales humanizados que utilizan modelos tridimensionales de las secuencias parentales y humanizadas. Modelos de inmunoglobulina tridimensionales están comúnmente disponibles y son familiares para aquellos expertos en la técnica. Programas de computador están disponibles que ilustran y exhiben probables estructuras con formación tridimensional de secuencias de inmunoglobulina candidatas seleccionadas. La inspección de estas exhibiciones permite el análisis del papel probable de los residuos en el funcionamiento de la secuencia de inmunoglobulina candidato, es decir, el análisis de residuos que influencian la capacidad de la inmunoglobulina candidato para unir su antígeno. De esta forma, los residuos FR se pueden seleccionar y combinar a partir del recipiente y las secuencias importantes de tal forma que la característica de anticuerpo deseado, tal como incremento de afinidad para el antígeno objetivo, se logra. En general, los residuos de la región hipervariable están directa y más sustancialmente involucrados en influenciar la unión de antígeno.

(iv) Anticuerpos humanos

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Como una alternativa a la humanización, de anticuerpos humanos, se pueden generar. Por ejemplo, ahora es posible producir animales transgénicos (por ejemplo, ratones) que son capaces, luego de inmunización, producir un completo repertorio de anticuerpos humanos en la ausencia de la producción de inmunoglobulina endógena. Por ejemplo, se ha descrito que la eliminación homocigota del gen (J_H) de la región unión de cadena pesada de anticuerpo en ratones mutantes de línea germinal y quimérica resulta en la inhibición completa de la producción de anticuerpo endógeno. La transferencia del ensayo de gen de inmunoglobulina de línea germinal humana en tales ratones mutantes de línea germinal resultara en la producción de anticuerpos humanos luego de inoculación del antígeno. Ver, 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); y patentes estadounidenses números. 5,591,669, 5,589,369 y 5,545,807.

Alternativamente, la tecnología de exhibición de fago (McCafferty *et al.*, *Nature* 348:552-553 (1990)) se puede utilizar para producir anticuerpos humanos i fragmentos de anticuerpos *in vitro*, a partir de repertorios de gen de dominio (V) variable de inmunoglobulina a partir de donantes no inmunizados. De acuerdo con esta técnica, los genes de dominio V de anticuerpo se clonan en estructura en un gen de proteína de cubierta mayor o menor de una bacteriófago filamentoso, tal como M13 o fd, y exhibidos como fragmentos de anticuerpo funcional en la superficie de la

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partícula fago. Debido a que la partícula filamentosa contiene una copia de ADN de hebra sencilla del genoma fago, selecciones basadas en las propiedades funcionales del anticuerpo también resulta en la selección del gen que codifica el anticuerpo que exhibe estas propiedades. Así, el fago imita alguna de las propiedades de la célula B. la exhibición del fago se puede desarrollar en una variedad de formatos; para su revisión ver, por ejemplo, Johnson, Kevin S. y Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Varias fuentes de segmento de gen V se pueden utilizar para exhibir fago. Clackson *et al., Nature,* 352:624-628 (1991) aísla un ensayo diverso de anticuerpos anti-oxazolona a partir de una librería combinatoria aleatoria pequeña genes V derivados de bazos de ratones inmunizados. Un repertorio de genes V genes de donantes humanos no inmunizados se puede construir y los anticuerpos para un ensayo diverso de antígenos (que incluyen auto-antígenos) se puede aislar esencialmente siguiendo las técnicas descritas por Marks *et al., J. Mol. Biol.* 222.581-597 (1991), o Griffith *et al., EMBO J.* 12:725-734 (1993). Ver, también, patentes estadounidense números 5,565,332 y 5,573,905.

También se pueden generar anticuerpos humanos mediante células B activadas *in vitro* (ver patentes estadounidenses 5,567,610 y 5,229,275).

(v) Fragmentos de anticuerpo

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Se han desarrollado varias técnicas para la producción de fragmento de anticuerpo. Tradicionalmente, estos fragmentos se derivan por vía de la digestión proteolítica de anticuerpos intactos (ver, 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 se pueden producir ahora directamente mediante células huésped recombinantes. Por ejemplo, los fragmentos de anticuerpo se pueden aislar a partir de librerías fago de anticuerpos discutidos anteriormente. Alternativamente, los fragmentos, Fab'-SH se pueden recubrir directamente a partir de *E. coli* y acoplar químicamente para formar fragmentos $F(ab')_2$ (Carter *et al.*, Bio/Technology 10:163-167 (1992)). De acuerdo con otra aproximación, se pueden aislar los fragmentos $F(ab')_2$ directamente a partir del cultivo de células huésped recombinantes. Otras técnicas para la producción de fragmentos de anticuerpos serán evidentes para el practicante experto. En otras modalidades, el anticuerpo de elección es un fragmento Fv de cadena sencilla (scFv). Ver WO 93/16185; Patente estadounidense número 5,571,894; y Patente estadounidense número 5,587,458. El fragmento de anticuerpo también puede ser un "anticuerpo lineal", por ejemplo, como se describe en la Patente estadounidense 5,641,870 por ejemplo. Tales fragmentos de anticuerpos lineales pueden monoespecíficos o biespecíficos.

(vi) Anticuerpos biespecíficos

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Los anticuerpos biespecíficos son anticuerpos que tiene especificidad de unión para al menos dos epítopos diferentes. Anticuerpos biespecíficos de ejemplo pueden unirse a dos epítopos diferentes de el marcador de superficie de célula B. otros de tales anticuerpos pueden unir el marcador de superficie de célula B y unir adicionalmente un segundo marcador de superficie de célula B diferente. Alternativamente, un marcador de superficie de célula anti-B que une un brazo se puede combinar con un brazo que une una molécula disparadora en un leucocito tal como una molécula receptora de célula T (por ejemplo CD2 o CD3), o receptores Fc para IgG (FcγR), tal como FcγRI (CD64), FcγRII (CD32) y FcγRIII (CD16) de tal forma que enfoca mecanismos de defensa celular para la célula B. Anticuerpos biespecíficos también se pueden utilizar para localizar agentes citotóxicos para la célula B. estos anticuerpos poseen un brazo de unión de marcador de superficie de célula B y un brazo que une el agente citotóxico (por ejemplo saporina, anti-interferón-α, alcaloide vinca, cadena de ricino A, metotrexato o hapteno de isótopo radioactivo). Anticuerpos biespecíficos se pueden preparar como anticuerpos de longitud completa o fragmentos de anticuerpo (por ejemplo anticuerpos biespecíficos $F(ab')_2$).

Métodos para la elaboración de anticuerpos biespecíficos son conocidos en la técnica. La producción tradicional de anticuerpos biespecíficos de longitud completa se basa en la coexpresión de dos pares de cadena liviana-cadena pesada de inmunoglobulina, en donde las dos cadenas tienen especificidades diferentes

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(Millstein *et al.*, *Nature*, 305:537-539 (1983)). Debido a la clasificación aleatoria de las cadenas liviana y pesada de inmunoglobulina, estos hibridomas (quadromas) producen una mezcla potencial de 10 diferentes moléculas de anticuerpo, de las cuales solo una tiene la estructura biespecífica correcta. La purificación de la molécula correcta, que usualmente se hace por etapas de cromatografía de afinidad, es bastante incomodo, y los rendimientos de producto son bajos. Procedimientos similares se describen en la WO 93/08829, y en Traunecker *et al*, *EMBO J.*, 10:3655-3659 (1991).

De acuerdo con una aproximación diferente, los dominios variables de anticuerpo con las especificidades de unión deseadas (sitios de combinación de anticuerpo-antígeno) se fusionan con secuencias de dominio constantes de inmunoglobulina. La fusión preferiblemente está con un dominio constante de cadena pesada de inmunoglobulina que comprende al menos parte de las regiones de articulación, CH2, y CH3. Se prefiere tener la primera región constante de cadena pesada (CH1) que contiene el sitio necesario para unir la cadena liviana, presente en al menos una de las fusiones. El ADN que codifica las fusiones de cadena pesada de inmunoglobulina y, si se desea, la cadena liviana de inmunoglobulina, se insertan en vectores de expresión separados, y se cotransfieren en organismos huéspedes adecuados. Esta suministra gran flexibilidad en el ajuste de proporciones mutuas de los tres fragmentos de polipéptido en modalidades cuando proporciones no iguales de las tres cadenas de polipéptido

utilizadas en la construcción suministran los rendimientos óptimos. Es, sin embargo, posible insertar las secuencias de codificación para dos o todas las tres cadenas de polipéptido en un vector de expresión cuando la expresión de al menos dos cadenas de polipéptido en proporciones iguales resulta en altos rendimientos o cuando las proporciones no son de particular significancia.

En una modalidad preferida de esta aproximación, los anticuerpos biespecíficos están compuestos de una cadena pesada de inmunoglobulina hibrida con una primer especificidad de unión en un brazo, y un par de cadena liviana-pesada de inmunoglobulina hibrida (que suministra una segunda especificidad de unión) en el otro brazo. Se encuentra que esta estructura asimétrica facilita la separación del compuesto biespecífico de combinaciones de cadena inmunoglobulina no deseadas, como la presencia de una cadena liviana de inmunoglobulina en solo una mitad de la molécula biespecífica suministra una vía fácil de separación. Esta aproximación se describe en la WO 94/04690. Para detalles adicionales de generación de anticuerpos biespecíficos ver, por ejemplo, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

De acuerdo con otra aproximación descrita en la Patente estadounidense número 5,731,168, la interfaz entre un par de moléculas de anticuerpo puede ser construida con ingeniería para maximizar el porcentaje de heterodímeros que están recubiertos a partir de cultivos de células recombinantes. La interfaz

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preferida comprende al menos una parte del dominio C_H3 de un dominio constante de anticuerpo. En este método, uno o más cadenas laterales de aminoácido pequeñas de la interfaz de la primer molécula de anticuerpo se reemplaza por cadenas laterales más grandes (por ejemplo tirosina o triptofan). "cavidades" compensatorias de tamaño similar o idéntico a las cadenas laterales grandes se crea en la interfaz de la segunda molécula de anticuerpo al reemplazar las cadenas laterales de aminoácido grandes con unas más pequeñas (por ejemplo alanina o treonina). Esto suministra un mecanismo para incrementar el rendimiento del heterodímero sobre otros productos finales no deseados tales como homodímeros.

Anticuerpos biespecíficos incluyen anticuerpos "heteroconjugados" o reticulados. Por ejemplo, uno de los anticuerpos en el heteroconjugado se puede acoplar con avidina, el otro con biotina. Tales anticuerpos han sido, por ejemplo, propuestos para células de sistema inmunes objetivo para células indeseadas (Patente estadounidense número 4,676,980), y para tratamiento de infección por VIH (WO 91/00360, WO 92/200373, y EP 03089).

Anticuerpos heteroconjugados se pueden hacer utilizando cualesquier métodos de reticulación. Los agentes de reticulación adecuados bien conocidos en la técnica, se describen en la patente estadounidense número 4,676,980, junto con un número de técnicas de reticulación.

Las técnicas para generar anticuerpos biespecíficos a partir de fragmentos de anticuerpo también se han descrito en la literatura. Por ejemplo, anticuerpos biespecíficos se pueden preparar utilizando enlaces químicos. Brennan *et al.*, *Science*, 229: 81 (1985) describe un procedimiento en donde los anticuerpos intactos se clivan proteolíticamente para generar fragmentos $F(ab')_2$. Estos fragmentos se reducen en la presencia de arsenita de sodio agente complejante ditiol para estabilizar ditioles vecinos y evitar la formación de disulfuro intramolecular. Los fragmentos Fab' generados se convierten luego a derivados de tionitrobenzoato (TNB). Unos de los derivados Fab'-TNB se reconvierte luego al Fab'-tiol mediante reducción con mercaptoetilamina y se mezcla con una cantidad equimolar del otro derivado Fab'-TNB para formar el anticuerpo biespecífico. Los anticuerpos biespecíficos producidos se pueden utilizar como agentes para la inmovilización selectiva de enzimas.

Varias técnicas para elaborar y aislar fragmentos de anticuerpo biespecíficos directamente a partir de cultivos de células recombinantes también se ha descrito. Por ejemplo, anticuerpos biespecíficos se han producido utilizando 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 enlazan a las porciones Fab' de dos anticuerpos diferentes mediante fusión de gen. Los homodímeros de anticuerpo se calculan en la región de articulación para formar monómeros y luego

reoxidarlos para formar los heterodímeros de anticuerpo. Este método también se puede utilizar para la producción homodímeros de anticuerpo. La tecnología de "fragmento divalente" descrita por Hollinger *et al., Proc. Natl. Acad. Sci.* USA, 90:6444-6448 (1993) ha suministrado un mecanismo alternativo para elaborar fragmentos de anticuerpo biespecíficos. Los fragmentos comprenden un dominio variable de cadena pesada (V_H) conectado con un dominio variable de cadena liviana (V_L) mediante un enlace que es muy corto para permitir el acoplamiento entre los dos dominios de la misma cadena. De acuerdo con esto, los dominios V_H y V_L de un fragmento se forzan a acoplarse con los dominios complementarios V_L y V_H de otro fragmento, formando por lo tanto dos sitios de unión de antígeno. Otra estrategia para elaborar fragmentos de anticuerpo biespecíficos mediante el uso de dímeros Fv (sFv) de cadena sencilla también se ha reportado. Ver Gruber *et al., J. Immunol*, 152:5368 (1994).

Anticuerpos con más de dos valencias se contemplan. Por ejemplo se pueden preparar anticuerpos triespecíficos. Tutt *et al., J. Immunol.* 147: 60 (1991).

IV. Conjugados y otras modificaciones del anticuerpo

El anticuerpo utilizado en los métodos o incluido en los artículos de fabricación aquí se conjuga opcionalmente con un agente citotóxico. Por ejemplo, el anticuerpo se puede conjugar con una droga como se describe en WO2004/032828.

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Agentes quimioterapéuticos útiles en la generación de tales conjugados de agentes de anticuerpos citotóxico se ha descrito anteriormente.

Conjugados de un anticuerpo y una o más toxinas de molécula pequeña, tal como caliqueamicina, una maitansina (Patente estadounidense número 5,208,020), un tricoteno y CC1065 también se contemplan aquí. En una modalidad de la invención, el anticuerpo se conjuga con una o más moléculas de maitansina (por ejemplo aproximadamente 1 a aproximadamente 10 moléculas de maitansina por molécula de anticuerpo). La maitansina puede, por ejemplo, ser convertida a May-SS-Me, que se puede reducir a May-SH3 y reaccionar con el anticuerpo modificado (Chari *et al. Cancer Research* 52:127-131 (1992)) para generar un conjugado de anticuerpo maitansinoide.

Alternativamente, el anticuerpo se conjuga con una o más moléculas de caliqueamicina. La familia caliqueamicina de antibióticos son capaces de producir ADN bicantenario en concentraciones sub-picomolares. Análogos estructurales de caliqueamicina que se pueden utilizar incluyen, pero no están limitadas a, γ_1^I, α_2^I, α_3^I, N-acetil-γ_1^I, PSAG y θ^I_1 (Hinman *et al. Cancer Research* 53:3336-3342 (1993) y Lode *et al., Cancer Research* 58:2925-2928 (1998)).

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Toxinas y fragmentos activos enzimáticamente de estos que se pueden utilizar incluyen cadena A difteria, fragmentos activos sin unión de toxina difteria, cadena A exotoxina (de *Pseudomonas aeruginosa*), cadena A ricino, cadena A abrina, cadena A modesina, alfa-sarcin, proteínas *Aleurites fordii*, proteínas diantina, proteinas *Fitolaca americana* (PAPI, PAPII, y PAP-S), inhibidor de charantia momordica, curcina, crotina, inhibidor de sapaonaria officinalis, gelonina, mitogelina, restrictocina, fenomicina, enomicina y los tricotecenos. Ver, por ejemplo, WO 93/21232 publicado en octubre 28, 1993.

La presente invención contempla adicionalmente anticuerpos conjugados con un compuesto con actividad nucleolítica (por ejemplo una ribonucleasa o una endonucleasa de ADN tal como deoxiribonucleasa; DNasa).

Una variedad de isótopos radioactivos están disponibles para la producción de anticuerpos randioconjugados. 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 de anticuerpo y agentes citotóxicos se pueden hacer utilizando una variedad de agentes de acoplamiento de proteína bifuncional tales como N-succinimidil-3-(2-piridilditiol) propionato (SPDP), succinimidil-4-(N-maleimidometil) ciclohexano-1-carboxilato, iminotiolano (IT), derivados bifuncionales de imidoesteres (tal como HCL adipimidato

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dimetilo), esteres activos (tal como suberato de disuccinimidilo), aldehídos (tal como glutaraldehidos), compuestos bis-azido (tal como bis (p-azidobenzoil) hexanodiamina), derivados de bis-diazonio (tal como bis-(p-diazoniumbenzoil)-etilenediamina), diisocianatos (tales como tolieno 2,6-diisocianato), y compuestos de flúor bis-activo (tal como 1,5-difluoro-2,4-dinitrobenzeno). Por ejemplo, se puede preparar una inmunotoxina ricino como se describe en Vitetta *et al. Science* 238:1098 (1987). El ácido Carbon-14-etiquetado 1-isotiocianatobenzil-3- metildietileno triaminopentaacetico (MX-DTPA) es un agente quelante de ejemplo para la conjugación de radionucleótido para el anticuerpo. Ver WO94/11026. El ligador puede ser un ligador "clivable" que facilita la liberación de una droga citotóxica en la célula. Por ejemplo, un ligador de acido-lábil, ligador sensible a la peptidasa, ligador dimetilo o ligador que contiene disulfuro (Chari *et al. Cancer Research* 52:127-131 (1992)) se puede utilizar.

Alternativamente, una proteína de fusión que comprende un agente citotóxico y anticuerpo se puede hacer, por ejemplo, mediante técnicas de recombinación o síntesis de péptido.

En aun otra modalidad, el anticuerpo se puede conjugar con un "receptor" (tal como estreptavidina) para la utilización en preobjetivar tumores en donde el conjugado de recetor-anticuerpo se administra al sujeto, seguido por la remoción de conjugado no unido a partir de la circulación utilizando agente clarificante y

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luego la administración de "ligando" (por ejemplo avidina) que se conjuga con un agente citotóxico (por ejemplo un radionucleótido).

Los anticuerpos de la presente invención también se pueden conjugar con enzima que activa prodroga que convierte una prodroga (por ejemplo un agente quimioterapéutico peptidilo, ver WO81/01145) a una droga anticáncer activa. Ver, por ejemplo, WO 88/07378 y Patente estadounidense número 4,975,278.

El componente de enzima de tales conjugados incluye cualquier enzima capaz de actuar sobre una prodroga en tal forma que la convierte en su forma citotóxica más activa.

Enzimas que son útiles en el método de esta invención incluyen, pero no están limitadas a, fosfatasa alcalina útil para convertir prodrogas que contienen fosfato en drogas libres; arilsulfatasa útil para convertir prodrogas que contienen sulfato en drogas libres; deaminasa citosina útil para convertir 5-fluorocitosina no toxica en la droga anticáncer, 5-fluorouracilo; proteasas, tal como serratia proteasa, termolisina, subtilisina, carboxipéptidasas y catepsinas (tal como catepsinas B y L), que son útiles para convertir prodrogas que contienen péptido en drogas libres; D-alanilcarboxipeptidasas, útiles para convertir prodrogas que contienen sustituyentes aminoácido-D; enzimas que clivan carbohidrato tal como β-galactosidasa y neuraminidasa utiles para convertir prodrogas glicosiladas en drogas libres; β-

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lactamasa útil para convertir drogas derivadas con β-lactamas en drogas libres; y amidasas penicilina, tal como penicilina V amidasa o penicilina G amidasa, útil para convertir drogas derivadas en sus nitrógenos amina con grupos fenoxiacetilo o fenilacetilo, respectivamente, en drogas libres. Alternativamente, los anticuerpos con actividad enzimática, también conocidos en la técnica como "abismas", se pueden utilizar para convertir las prodrogas de la invención en drogas activas libres (ver, por ejemplo, *Massey, Nature* 328:457-458 (1987)). Los conjugados de anticuerpo abzima se pueden preparar como se describe aquí para el suministro de la abzima a una población de célula de tumor.

Las enzimas de esta invención se pueden unir covalentemente al anticuerpo mediante técnica bien conocidas en el arte tal como el uso de reactivos de reticulación heterobifuncionales discutidos anteriormente. Alternativamente, las proteínas de fusión que comprenden al menos la región de unión de antígeno de un anticuerpo de la invención ligada al menos a una porción activa funcionalmente de una enzima de la invención se puede construir utilizando técnica de ADN recombinantes bien conocidas en la técnica (ver, por ejemplo , Neuberger *et al.*, *Nature*, 312:604-608 (1984)).

Otras modificaciones del anticuerpo se contemplan aquí. Por ejemplo, el anticuerpo se puede ligar a una de una variedad de polímeros no proteináceos, por ejemplo, polietilenglicol (PEG),

polipropilenglicol, polioxialquilenos, o copolímeros de polietilenglicol y polipropilenglicol. Fragmentos de anticuerpo, tal como Fab', ligado a una o más moléculas PEG que son una modalidad especialmente preferida de la invención.

Los anticuerpos descritos aquí también se pueden formular como liposomas. Los liposomas que contienen el anticuerpo se preparan por métodos conocidos en la técnica, tal como se describe en Epstein *et al., Proc. Natl. Acad. Sci.* USA, 82:3688 (1985); Hwang *et al, Proc. Natl. Acad. Sic.* USA, 77:4030 (1980); Patente estadounidense números 4,485,045 y 4,544,545; y WO97/38731 publicada en octubre 23, 1997. Liposomas con tiempo de circulación mejorada se describen en la patente estadounidense número 5,013,556.

Liposomas particularmente útiles se pueden generar mediante el método de evaporación de fase reversa con una composición de lípido que comprende fosfatidilcolina, colesterol y fosfatidiletanolamina derivada PEG (PEG-PE). Los liposomas se extruyen a través de filtros de tamaño de poro definido para producir liposomas con el diámetro deseado. Fragmentos Fab' de un anticuerpo de la presente invención se pueden conjugar con los liposomas como se describe en Martin *et al. J. Biol. Chem.* 257:286-288 (1982) por vía de una reacción de intercambio de disulfuro. Un agente quimioterapéutico está opcionalmente contenido dentro del liposoma. Ver Gabizon *et al. J. National Cancer Inst.* 81(19)1484 (1989).

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Modificaciones de secuencia de aminoácido del anticuerpo se contemplan. Por ejemplo, puede ser deseable mejorar la afinidad de unión y/o otras propiedades biológicas del anticuerpo. Variantes de la secuencia de aminoácido del anticuerpo se preparan al introducir cambios de nucleótido apropiado en el ácido nucleico del anticuerpo, o mediante síntesis de péptido. Tales modificaciones incluyen, por ejemplo, eliminaciones de, y/o inserciones en y/o sustituciones de, residuos dentro de la secuencia de aminoácido del anticuerpo. Cualquier combinación de eliminación, inserción y sustitución se hacen para llevar en la construcción final, dado que la construcción final posee las características deseadas. Los cambios de aminoácido también pueden alterar los procesos post-transduccionales del anticuerpo, tal como cambiar el número o posesión de los sitios de glicosilación.

Un método útil para la identificación de ciertos residuos o regiones del anticuerpo que son ubicaciones preferida para mutagénesis se llama "mutagénesis de exploración de alanina" como se describe por Cunningham and Wells *Science*, 244:1081-1085 (1989). Aquí, un residuo o grupo de residuos objetivo se identifica (por ejemplo, residuos cargados tal como arg, asp, his, lys, y glu) y reemplazados por un aminoácido cargado negativamente o neutral (mas preferiblemente alanina o polialanina) para afectar la interacción de los aminoácidos con antígeno. Aquellas ubicaciones de aminoácido que demuestran

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sensibilidad funcional para las sustituciones luego se refinan al introducir adicionalmente u otras variantes en, o para, los sitios de sustitución. Así, mientras es sitio para introducir una variación de secuencia de aminoácido se predetermina, la naturaleza de la mutación *per se* no necesita ser predeterminada. Por ejemplo, para analizar el desempeño de una mutación en un sitio dado, la exploración ala o mutagénesis aleatoria se conduce en el codón objetivo o región y las variantes de anticuerpo expresadas se seleccionan para la actividad deseada.

Las inserciones de secuencia de aminoácidos incluyen fusiones amino- y/o carboxilo-terminales que varían en la longitud desde un residuo a polipéptidos que contienen cien o más residuos, así como también inserciones de intrasecuencia de residuos de aminoácidos sencillos o múltiples. 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 del terminal N- o C- del anticuerpo de una enzima, o un polipéptido que incrementa la vida media del suero del anticuerpo.

Otro tipo de variante es una variante de sustitución de aminoácido. Estas variantes tienen al menos un residuo aminoácido en la molécula de anticuerpo reemplazada por diferentes residuos. Los sitios de mayor interés para mutagénesis de sustitución de anticuerpos incluye las regiones hipervariables, pero también se

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contemplan alteraciones FR. Se muestran sustituciones conservadoras en la tabla 1 de acuerdo con el encabezado de "sustituciones preferidas". Si tales sustituciones resultan en un cambio en la actividad biológica, entonces más cambios sustanciales, denominados "sustituciones de ejemplos" en la tabla 1, o como descritas adicionalmente adelante con referencia a clases de aminoácidos, se pueden introducir y los productos seleccionar.

Tabla 1

	Ala; Norleucina	
Residuo Original	Sustituciones de Ejemplo	Sustituciones Preferidas
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn(N)	Gln; Bis; 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
Bis (B)	Asn; Gln; Lys; Arg	Arg
Ile(I)	Leu; Val; Met; Ala; Phe; Norleucina	Leu
Leu (L)	Norleucina; IIe; 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;	Leu

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Modificaciones sustanciales en las propiedades biológicas del anticuerpo se realizan al seleccionar sustituciones que difieren significativamente en su efecto al mantener (a) de la estructura del polipéptido en el área de sustitución, por ejemplo, como una hoja o con formación helicoidal, (b) la carga o hidrofobicidad de la molécula en el sitio objetivo, o (c) la masa de la cadena lateral. Los aminoácidos se pueden agrupar de acuerdo con similitudes en las propiedades de sus cadenas laterales (en A. L. Lehninger, en *Biochemistry*, segunda ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) no-polar: Ala (A), VaI (V), Leu (L), He (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) polar no cargado: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), GIn (Q)

(3) acídico: Asp (D), GIu (E)

(4) básico: Lys (K), Arg (R), His(H)

Alternativamente, los residuos de ocurrencia natural se pueden dividir en grupos basados en propiedades de cadena lateral común:

(1) hidrófobo: Norleucine, Met, Ala, VaI, Leu, He;

(2) hidrofilico neutral: Cys, Ser, Thr, Asn, GIn;

(3) acídico: Asp, GIu;

(4) básico: His, Lys, Arg;

(5) residuos que influencian la orientación de cadena: GIy, Pro;

(6) aromático: Trp, Tyr, Phe.

Sustituciones no conservadoras traerán consigo el intercambio de un miembro de una de esta clase por otra clase.

Cualquier residuo de cisteína no involucrado en mantener la conformación apropiada del anticuerpo también se puede sustituir, generalmente con serina. Para mejorar la estabilidad oxidativa de la molécula y evitar reticulación aberrante. Por el contrario, los enlaces de cisteína se pueden agregar al anticuerpo para mejorar su estabilidad (particularmente cuando el anticuerpo es un fragmento de anticuerpo tal como fragmento Fv).

Un tipo particularmente preferido de variantes sustitucionales involucra sustituir uno o más residuos de región hipervariable de un anticuerpo pariente. Generalmente, las variantes resultantes seleccionadas para desarrollo adicional tendrán propiedades biológicas mejoradas relacionadas con el anticuerpo pariente del cual ellas se generan. Una forma conveniente para generar tales variantes de sustitución es la maduración de afinidad utilizando la exhibición de fago. Resumen, varios sitios de región hipervariable (por ejemplo, 6-7 sitios) se mutan para generar todas las sustituciones aminos posibles en cada sitio. Las variantes de anticuerpo generadas así se exhiben en una forma monovalente a partir de partículas de fago filamentosas como fusiones al producto de gen III del M13 empacado dentro de cada partícula. Las variantes fago desplegadas

luego se seleccionan luego por su actividad biológica (por ejemplo afinidad de unión) como se describe aquí. Con el fin de identificar sitios de región hipervariable candidato para modificación, se puede desarrollar mutagénesis de exploración de alanina para identificar los residuos de región hipervariable que contribuyen significativamente a la unión de antígeno. Alternativamente, o adicionalmente, esto puede ser beneficio para analizar una estructura cristal del complejo de anticuerpo antígeno para identificar puntos de contacto entre el anticuerpo y el antígeno. Tales residuos de de contacto y residuos vecinos son candidatos para sustitución de acuerdo con técnicas elaboradas aquí. Una de tales variantes se genera, el panel de variantes se somete a selección como se describe aquí y los anticuerpos con propiedades superiores en uno o más ensayos relevantes se pueden seleccionar para desarrollo adicional.

Otro tipo de variante de aminoácido del anticuerpo altera el patrón de glicosilación original del anticuerpo. Tal alteración incluye eliminar uno o más porciones de carbohidratos encontrados en el anticuerpo, y/o agregar uno o más sitios de glicosilación que no están presentes en el anticuerpo.

La gliciosilación de polipéptidos es típicamente N-enlazada o O-enlazada. N-enlazada se refiere a la unión de la porción carbohidratos a la cadena lateral de un residuo asparagina. La secuencias tripéptido asparagina-X-serina y asparagina-X-treonina, en donde X es cualquier aminoácido excepto prolina, son

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las secuencias de reconocimiento para unión enzimático de la porción carbohidratos a la cadena lateral asparagina. Así, la presencia de cualquiera de estas secuencias tripéptido en un polipéptido crea un sitio de glicosilación potencial. La glicosilación O-enlazada se refiere a la unión de unos de los azucares N-aceilgalactosamina, galactosa, o xilosa a un hidroxiamino acido, más comúnmente serina o treonina, aunque también se puede utilizar 5-hidroxiprolina o 5-hidroxilisina.

La adición de sitios de glicosilación al anticuerpo se logra convenientemente al alterar la secuencia de aminoácido de tal forma que esta contiene uno o más de las secuencias tripéptido descritas anteriormente (para sitios de glicosilación N-enlazados). La alteración también puede ser hecha mediante la adición de, o sustitución de, uno o más residuos serina o treonina a la secuencia del anticuerpo original (sitio de glicosilación O-enlazados).

En donde el anticuerpo comprende una región Fc, el carbohidrato adherido al este se puede alterar. Por ejemplo, los anticuerpos con una estructura carbohidratos madura que les falta fucosa unida a una región Fc del anticuerpo se describen en la solicitud de patente estadounidense número US 2003/0157108 A1,Presta,L. Ver también US 2004/0093621 A1 (Kyowa Hakko Kogyo Co., Ltd) relacionada con una composición de anticuerpo CD20. Los anticuerpos con una bisección N-acetilglucosamina (GlcNAc) en el carbohidratos adherido a una región Fc del anticuerpo se

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referencian en la WO03/011878, Jean-Mairet *et al.* y Patente estadounidense número 6,602,684, Umana *et al.* A anticuerpos con al menos un residuo galactosa en el oligosacárido unido a una región Fc del anticuerpo se reportan en la WO97/30087, Patel *et al.* Ver, también, WO98/58964 (Raju, S.) y WO99/22764 (Raju, S.) que se relacionan con anticuerpos con carbohidratos unido alterado a la región Fc de este.

La variante de glicosilación preferida aquí comprende una región Fc, en donde una estructura de carbohidratos unida a la región Fc le falta fucosa. Tales variantes tienen función ADCC mejorada. Opcionalmente, la región Fc comprende adicionalmente una o más sustituciones de aminoácido en esta que mejoran adicionalmente el ADCC, por ejemplo, las sustituciones ne las posiciones 298,333, y/o 334 de la región Fc (numeración Eu de residuos). Ejemplos de publicaciones relacionadas con anticuerpos "defucosilado" o "fucosa-deficiente" incluyen: solicitud de patente estadounidense número US 2003/0157108 A1, Presta,L; WO 00/61739A1; WO01/29246A1; US2003/0115614A1; US2002/0164328A1; US2004/0093621A1; US2004/0132140A1; US2004/0110704A1; US2004/0110282A1; US2004/0109865A1; WO03/085119A1; WO03/084570A1; WO2005/035778; WO2005/035586 (que describe la inhibición del ARN (ARNi) de fucosilación); Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87:614 (2004). Ejemplos de líneas celulares que producen anticuerpos defucosilados incluyen células Lec13 CHO deficientes de fucosilación de proteína (Ripka *et al. Arch. Biochem. Biophys.*

249:533-545 (1986); solicitud de patentes estadounidense número US 2003/0157108 A1, Presta,L; y WO 2004/056312 A1, Adams *et al.*, especialmente en el ejemplo 11), y líneas celulares mutantes, tal como el gen alfa-1,6-fucosiltransferasa, FUT8, células CHO mutantes (Yamane-Ohnuki *et al.* Biotech. Bioeng. 87:614 (2004)).

Las moléculas de ácido nucleico que codifican variantes de secuencia de aminoácido del anticuerpo se preparan mediante una variedad de métodos conocidos en la técnica. Estos métodos incluyen, pero no están limitados a, aislamiento de una fuente natural (en el caso de variantes de secuencia de aminoácido de ocurrencia natural) o preparación por mutagénesis (o dirigida al sitio) mediada por oligonucleótido, mutagénesis PCR, y mutagénesis de casete de una variante preparada anteriormente o una versión no variante del anticuerpo.

Puede ser deseable modificar el anticuerpo de la invención con respecto a la función efectora, por ejemplo de tal forma que mejora la citotoxicidad mediada por célula dependiente de antígeno (ADCC) y/o citotoxicidad dependiente de complemento (CDC) del anticuerpo. Esta se puede alcanzar al introducir una o más sustituciones de aminoácido en una región Fc de un anticuerpo. Alternativa o adicionalmente, el residuo cisteína se puede introducir en la región Fc, permitiendo por lo tanto la formación de enlace de disulfuro intercadena en esta región. El anticuerpo homodimérico generado así puede tener capacidad de internalización mejorada y/o muerte celular mediada por

complemento incrementada y citotoxicidad dependiente de celular dependiente de anticuerpo (ADCC). Ver Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) y Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Anticuerpos homodiméricos con actividad antitumor mejorada también se pueden preparar utilizando reticuladores heterobifuncionales como se describe Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternativamente, un anticuerpo se puede construir por ingeniería de tal forma que tenga regiones Fc duales y puede tener por lo tanto lisis de complemento mejorado y capacidades ADCC. Ver Stevenson *et al. Anti-Cancer DrugDesign* 3:219-230 (1989).

WO00/42072 (Presta, L.) describe anticuerpos con función ADCC mejorada en la presencia de células efectoras humanas, en donde los anticuerpos comprenden sustituciones de aminoácido en la región Fc de esta. Preferiblemente, el anticuerpo con ADCC mejorado comprende sustituciones en la posiciones 298, 333, y/o 334 de la región Fc. Preferiblemente la región Fc alterada es una región Fc IgG1 humana que comprende o que consiste 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 estadounidense números 6,194,551B1, Patente estadounidense número 6,242,195B1, Patente estadounidense número 6,528,624B1 y Patente estadounidense número 6,538,124 (Idusogie *et al.*). Los anticuerpos comprenden una sustitución de aminoácido

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en una o más posiciones de aminoácido 270, 322, 326, 327, 329, 313, 333 y/o 334 de la región Fc de esta.

Para incrementar la vida media del suero del anticuerpo, uno puede incorporar un epítopo de unión de receptor salvaje en el anticuerpo (especialmente un fragmento de anticuerpo) como se describe en la Patente estadounidense 5,739,277, por ejemplo. Como se utiliza aquí el término "epítopo de unión de receptor salvaje" 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 por incrementar la vida media del suero *in vivo* de la molécula IgG. Anticuerpos con sustituciones en una región Fc de esta y vida media de suero incrementada también se describe en la WO00/42072 (Presta, L.).

Anticuerpos construidos con ingeniería con tres o más sitios de unión de antígeno funcional (preferiblemente cuatro) también se contemplan (solicitud estadounidense número No. US2002/0004587 A1, Miller *et al.*).

V. Formulaciones Farmacéuticas

Las formulaciones terapéuticas de los anticuerpos utilizados con la presente invención se preparan para almacenamiento al mezclar un anticuerpo que tiene un grado de pureza deseado con portadores farmacéuticamente aceptables opcionales, excipientes o estabilizadores (*Remington's Pharmaceutical Sciences* decimasexta

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edición, Osol, A. Ed. (1980)), en la forma de formulaciones liofilizadas oligonucleótidos soluciones acuosas. Portadores aceptables, excipientes, o estabilizadores no son tóxicos para los recipientes en las dosis y concentraciones empleadas, e incluyen amortiguadores tales como fosfato, citrato, y otros ácidos orgánicos, antioxidantes que incluyen ácido ascórbico y metionina; preservativos (tales como cloruro de amonio octadecildimetilbenzilo; cloruro de hexametonio; cloruro de benzalkonio, cloruro de benzetonio; fenol, butilo o alcohol benzilo; alquil parabenos tales como metilo o propil parabeno; catecol; resorcinol; ciclohexanol; 3-pentanol; y m-cresol); polipéptidos de bajo peso molecular (menos de aproximadamente 10 residuos); proteínas tal como albúmina de suero, gelatina, o inmunoglobulinas; polímeros hidrofilicos tales como polivinilpirrolidona; aminoácidos tales como glicina, glutamina, asparagina, histidina, arginina, o lisina; monosacáridos, disacáridos, y otros carbohidratos que incluyen glucosa, manosa, o dextrinas; agente quelantes tal como EDTA; azucares tales como sucrosa, manitol, trehalosa o sorbitol; contra iones que forman sal tal como sodio; complejos de metal (por ejemplo complejos de Zn-proteína); y/o tensoactivos no iónicos tal como TWEEN™, PLURONICS™ o polietilenglicol (PEG).

Formulaciones de anticuerpo anti-CD20 de ejemplos se describen en la WO98/56418. Esta publicación describe una formulación multidosis liquida que comprende 40 mg/mL de Rituximab, 25 mM de acetato, 150 mM de trehalosa, 0.9% de bencil

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alcohol, 0.02% de polisorbato 20 en pH 5.0 que tiene una vida de de posito mínima de dos años de almacenamiento a 2-8° C. Otra formulación anti-CD20 de interés comprende 10 mg/mL de Rituximab en cloruro de sodio 9.0 mg/mL, 7.35 mg/mL de dihidrato de citrato de sodio, 0.7 mg/mL de polisorbato 80, y agua estéril para inyección, pH 6.5.

Formulaciones liofilizadas adaptadas para administración subcutánea se describen en la patente estadounidense número 6,267,958 (Andya *et al.*). Tales formulaciones liofilizadas se pueden reconstituir con un diluyente adecuado para una concentración de proteína alta y la formulación reconstituida se puede administrar subcutáneamente al mamífero a ser tratado aquí.

Formulas cristalizadas del anticuerpo o anticuerpo también se contemplan. Ver, por ejemplo, US 2002/0136719A1 (Shenoy *et al.*).

La formulación aquí también puede contener más de un compuesto activo según sea necesario para la indicación particular que se trata, preferiblemente aquella con actividades complementarias que no Afectan adversamente la una a la otra. Por ejemplo, puede ser deseable suministrar adicionalmente un agente citotóxico; agente quimioterapéutico; agente inmunosupresor; citoquina, antagonista de citoquina o anticuerpo; factor de crecimiento; hormona; integrina; antagonista de integrina o anticuerpo (por ejemplo un anticuerpo LFA-1 tal como

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efalizumab/RAPTIVA disponible comercialmente de Genentech, o un anticuerpo integrina alfa 4 tal como natalizumab/TYSABRI) disponible de Biogen); drogas de clase interferón tal como IFN-beta-1a (REBIF® y AVONEX®) o IFN-beta-1b (BETASERON®); un oligopéptido tal como acetato glatirámero (COPAXONE®); un agente citotóxico tal como mitoxantrona (NOVANTRONE®), metotrexato, ciclofosfamida, clorambucil, o azatioprina; inmunoglobulinas intravenosas (gamma globulina); drogas de eliminación de linfocito (por ejemplo, mitoxantrona, ciclofosfamida, Campath, anti-CD4, o cladribina); drogas inmunosupresoras de agotamiento no-linfocito (por ejemplo, mofetil micofenolato (MMF) o ciclosporina); droga reductora del colesterol de la clase "estatina"; estradiol; testosterona; terapia de reemplazo hormonal; droga que tata síntomas secundarios o relacionados con MS (por ejemplo, espasmos, incontinencia, dolor, fatiga); un inhibidor TNF; droga antirreumática que modifica la enfermedad (DMARD); droga antiinflamatoria no-esteroidal (NSAID); corticosteroides (por ejemplo metilprednisolona, prednisona, dexametasona, o glucorticoide); levotiroxina; ciclosporina A; análogo de somatastatina; antagonista de citoquina; anti-metabolito; agente inmunosupresor; antagonista de integrina o anticuerpo (por ejemplo un anticuerpo LFA-I, tal como efalizumab o un anticuerpo de integrina alpha 4 tal como natalizumab); u otro antagonista/anticuerpo de superficie de célula B; etcétera en la formulación. El tipo y cantidades efectivas de tales otros agentes depende, por ejemplo, de la cantidad del anticuerpo presente en la formulación, el tipo de esclerosis múltiple que se

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trata, y los parámetros clínicos de los sujetos. Estos se utilizan generalmente en algunas dosis y con rutas de administración como se utiliza aquí anteriormente o aproximadamente de 1 a 99% de la dosis empleada aquí.

Los ingredientes activos también se pueden atrapado en microcápsulas preparadas, por ejemplo, mediante técnicas de coacervación o mediante polimerización interfacial, por ejemplo, hidrosimetil celulosa o microcápsulas-gelatina y microcápsulas poli-(metilmetacilato), respectivamente, en sistemas de suministro de droga coloidal (por ejemplo, liposomas, microesferas de albúmina, microemulsiones, nano-partículas y nanocápsulas) o en macroemulsiones. Tales técnicas se describen en *Remington's Pharmaceutical Sciences* decimasexta edición, Osol, A. Ed. (1980).

Se pueden preparar preparaciones de liberación sostenida. Ejemplos adecuados de preparaciones de liberación sostenida incluyen matrices semipermeables de polímeros hidrófobos sólidos que contienen el anticuerpo, cuyas matrices están en la forma de artículos formados, por ejemplo películas, o microcápsulas. Ejemplos de matrices de liberación sostenida incluyen poliésteres, hidrogeles (por ejemplo, poli(2-hidroxietil-metacrilato), o poli(vinilalcohol)), polilactidas (patente estadounidense número 3,773,919), copolímeros de ácido L-glutámico y γ etil-L-glutamato, acetato de vinilo-etilenono degradable, con polímeros de ácido glicolico-ácido láctico

degradables tales como LUPRON DEPOT™ (microesferas inyectables compuestas de copolímero de ácido glicolico-ácido láctico y acetato de leuprolida), y ácido poli-D-(-)-3-hidroxibutírico.

Las formulaciones a ser utilizadas para la administración *in vivo* deben ser estériles. Esto se logra fácilmente por filtración a través de membrana de filtración estéril.

VI. Artículos de fabricación

En otra modalidad de la invención, un artículo de fabricación que contiene materiales útiles para el tratamiento de esclerosis múltiple descrita anteriormente se suministra. Preferiblemente, el artículo de fabricación comprende: (a) un contenedor que comprende una composición que comprende un anticuerpo que une a un marcador de superficie de célula B (por ejemplo un anticuerpo CD20) y un portador farmacéuticamente aceptable o diluyente dentro del contenedor; y (b) un inserto de paquete con instrucciones para administrar la composición a un sujeto que sufre de esclerosis múltiple al sujeto para suministrar una exposición de anticuerpo inicial de aproximadamente 0.5 a 4 gramos seguido por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se suministra hasta aproximadamente 16 a 60 semanas a partir de la exposición inicial; o instrucciones para administrar la composición a un sujeto que sufre de PPMS.

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El artículo de fabricación comprende un contenedor y una etiqueta o inserto de paquete en o asociado con el contenedor. Contenedores adecuados incluyen, por ejemplo, botellas, frascos, jeringas, etcétera. Los contenedores se pueden formar de una variedad de materiales tal como vidrio o plástico. El contenedor soporta o contiene una composición que es efectiva para tratar la esclerosis múltiple y puede tener un puerto de acceso estéril (por ejemplo el contenedor puede ser una bolsa de solución intravenosa o un frasco que tiene un tope hinchable mediante una aguja de inyección hipodérmica). Al menos un agente activo en la composición es el anticuerpo. La etiqueta o inserto de paquete indica que la composiciones utiliza para tratar esclerosis múltiple en un sujeto que sufre de esta con la guía especifica con respecto a cantidades de dosis e intervalos de anticuerpo y cualquier otra droga que se suministre. El artículo de fabricación puede adicionalmente comprender un segundo contenedor que comprende un amortiguador de dilución farmacéuticamente aceptable, tal como agua bacterioestática para inyección (BWFI), salina amortiguada con fosfato, solución de Ringer y solución dextrosa. El artículo de fabricación puede incluir adicionalmente otros materiales deseables desde el punto de vista del usuario y comercial, que incluyen otros amortiguadores, diluyentes, filtros, agujas, y jeringas.

Opcionalmente, el artículo de fabricación aquí comprende adicionalmente un contenedor que comprende un agente diferente al anticuerpo para tratamiento y adicionalmente comprende

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instrucciones sobre el tratamiento al mamífero con tal agente, tal agente preferiblemente es un agente quimioterapéutico o agente inmunosupresor, droga de la clase interferón tal como IFN-beta-1a (REBIF® y AVONEX®) o IFN-beta-1b (BETASERON®); un oligopéptido tal como un acetato de glatirámero (COPAXONE®); un agente citotóxico tal como mitoxantrona (NOVANTRONE®), metotrexato, ciclofosfamida, clorambucil, o azatioprina; inmunoglobulina intravenosa (gamma globulina); droga de agotamiento de linfocito (por ejemplo, mitoxantrona, ciclofosfamida, Campath, anti-CD4, o cladribina); droga inmunosupresora de agotamiento no-linfocito (por ejemplo, mofetil micofenolato (MMF) o ciclosporina); droga reductora del colesterol de la clase "estatina"; estradiol; terapia de reemplazo hormonal; droga que trata síntomas secundarios o relacionados con MS (por ejemplo, espasmos, incontinencia, dolor, fatiga); un inhibidor TNF; droga antirreumática que modifica la enfermedad (DMARD); droga antiinflamatoria no-esteroidal (NSAID); corticosteroides (por ejemplo metilprednisolona, prednisona, dexametasona, o glucorticoide); levotiroxina; ciclosporina A; análogo de somatastatina; citoquina o antagonista receptor de citoquina; anti-metabolito; agente inmunosupresor; antagonista de integrina o anticuerpo (por ejemplo un anticuerpo LFA-1, tal como efalizumab o un anticuerpo integrina alpha 4 tal como natalizumab); y otro anticuerpo marcador de superficie de célula B; etcétera.

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Detalles adicionales de la invención se ilustran mediante los siguientes ejemplos no limitantes. La descripción de todas las citaciones en la especificación se incorporan expresamente aquí como referencia.

EJEMPLO 1

TRATAMIENTO PARA ESCLEROSIS MÚLTIPLE PROGRESIVA PRIMARIA (PPMS)

Un sujeto con diagnostico de PPMS como se define por McDonald *et al. Ann Neurol* 50:121-7 (2001) se trata con un anticuerpo CD20 en este ejemplo.

Rituximab, disponible comercialmente de Genentech, se formula para administración IV como un producto estéril en 9.0 mg/mL de cloruro de sodio, 0.7 mg/mL de polisorbato 80, 7.35 mg/mL de dihidrato de citrato de sodio, y agua estéril para inyección (pH 6.5).

El primer curso de tratamiento consistirá de una dosis de 1 g intravenosos de Rituximab (IV) administrado en cada uno de los días 1 y 15. Los sujetos recibirán acetaminofen (1 g) y difenhidramina HCl (50 mg), o equivalente, por la boca 30-60 minutos antes del inicio de cada infusión.

Cursos posteriores de tratamiento se administraran partiendo la semana 24 (día 169), semana 48 (día 337), y semana 72 (día

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505). La segunda infusión del curso posterior de tratamiento será de 14 ± 1 día después de la primera infusión.

Sujetos que experimentan una primer recaída pueden recibir tratamiento de rescate con corticosteriodes IV u orales. Corticosteroides sistémicos se pueden administrar utilizando un régimen que no excede la exposición o duración de tratamiento apropiado de una recaída MS. Una recaída se define como todo lo siguiente:

- Una aparición aguda de una anormalidad neurológica que persiste durante al menos 24 horas
- Un cambio no atribuible a fiebre, infección, trauma, medicación concomitante, u otra etiología
- Un evento con cambio objetivo en el examen por investigador examinador cegado, que incluye un mínimo de cambio de un punto en 1 de las siguientes escalas FS: piramidal, nervio craneal, cerebelar, sensorial, visión, o marcha

El siguiente régimen de corticosteroides se puede utilizar: 1 g de IV metilprednisolona diariamente durante 3 días, seguido por 60 mg de prednisona diariamente durante 5 días, y reducir mediante 10-mg de incrementos cada día de aquí en adelante. Si el IV metilprednisolonano está disponible, entonces 150 mg se puede sustituir con IV dexametasona diariamente durante 3 días. Solo un curso de corticosteroides se debe administrar para una

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exacerbación. Estudios inmunológicos posteriores y exploraciones MRI se deben obtener al menos 4 semanas después de la terminación del régimen de corticosteroides. Inhaladores de corticosteroide (oral y nasal) o inyecciones intra-articulares se pueden utilizar.

Tratamientos adicionales para ser opcionalmente combinados con el anticuerpo CD20 incluyen IFN-beta, acetato de glatirámero, metotrexato, ciclofosfamida, o mitoxantrona.

La medición del resultado de eficacia primaria es el tiempo para progresión de enfermedad confirmada. La progresión de enfermedad se define como un incremento de ≥ 1.0 puntos a partir de la Escala Ampliada Para Determinar el Estado de Incapacidad (EDSS) (Kurtzke J. *Neurologi* 33(11):1444-52 (1983)), si el EDSS de línea base está entre 2.0 y 5.5 puntos (inclusive), o un incremento de ≥ 0.5 puntos si el EDSS de línea base es ≥ 5.5 puntos (inclusive), para cuyo cambio no es atribuible a otra etiología (por ejemplo, fiebre, enfermedad concurrente, recaída MS o exacerbación, o medicación concomitante). La confirmación de la progresión de la enfermedad puede ocurrir en una visita programada regularmente que es de al menos 12 semanas (84 días) después de la progresión inicial.

Las medidas de resultado de eficacia secundarias incluyen:

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- Cambio de la línea base a la semana 96 en el volumen total de lesiones T2 en exploración MRI de cerebro
- Cambio de la línea base de la semana 96 en volumen de cerebro en exploración MRI de cerebro

Opcionalmente, la mejora en una cualquiera o más de:

- Escala Compuesta Funcional de Esclerosis Múltiple (MSFCS)
- EDSS
- Proporción de sujetos con progresión de enfermedad confirmada en la semana 96, según se determina utilizando EDSS
- Función de extremidad superior, según se mide por el Ensayo Peg de 9 Agujeros (una subescala del MSFCS)
- Ambulación, según se mide por el tiempo caminado de 25 pies (una subescala del MSFCS)
- Cognición, según se mide por la Prueba de Adición Auditiva Consecutiva Ritmada (solo 3 segundos; una subescala del MSFCS)
- Volumen total de lesiones T2 de cerebro en exploraciones MRI (semanas 48 y 122)
- Volumen total de lesiones T1 de cerebro en exploraciones MRI
- Área de sección cruzada de la medula espinal cervical en exploraciones MRI

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- Volumen de cerebro en exploraciones MRI (semanas 48 y 122)

El sujeto tratado con Rituximab como se describe aquí mostrara mejora en los signos, síntomas u otros indicadores de PPMS de acuerdo con una cualquiera o más de las medidas resultantes anteriores.

EJEMPLO 2

TRATAMIENTO DE ESCLEROSIS MÚLTIPLE RECIDIVANTE-REMISIÓN

Sujetos con RRMS como se define por McDonald *et al. Ann Neurol* 50:121-7 (2001) se tratan con anticuerpo CD20 en este ejemplo, en donde la exposición del es de aproximadamente 6 meses.

Rituximab, disponible comercialmente de Genentech, se formula para administración IV como un producto estéril en 9.0 mg/mL de cloruro de sodio, 0.7 mg/mL de polisorbato 80, 7.35 mg/mL de citrato de sodio deshidratado, y Agua Estéril para Inyección (pH 6.5).

El primer curso de tratamiento consistirá de una dosis de 1 g de Rituximab (IV) intravenoso administrado en cada uno de los días 1 y 15. Los sujetos recibirán acetaminofén (1 g) y difenhidramina HCl (50 mg), o equivalente, por la boca 30-60 minutos antes del inicio de cada infusión.

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Cursos posteriores de tratamiento se administraran partiendo de la semana 24 (día 169), semana 48 (dia 337), y semana 72 (día 505). La segunda infusión del curso posterior de tratamiento será de 14 ± 1 días después de la primera infusión.

Preferiblemente el Rituximab es el único agente administrado para tratar la RRMS. Sin embargo, los sujetos pueden opcionalmente recibir IV o corticosteroides orales, IFN-beta, acetato de glatirámero, metotrexato, ciclofosfamida, o mitoxantrona.

Sujetos que experimentan una primer recaída puede recibir tratamiento de rescate con IV o corticosteroides orales. Corticosteroides sistémicos se pueden administrar utilizando un régimen que no excede la exposición o duración del tratamiento apropiado para una recaída MS. Una recaída en este ejemplo se define como:

La aparición de nuevos síntomas o síntomas neurológicos recurrentes consistentes con el último MS más de 48 horas en un sujeto quien a sido relativamente estable o en estado de mejora neurológica durante al menos 30 días. El cambio en los síntomas neurológicos se debe acompañar por empeoramiento neurológico objetivo consistente con un incremento de al menos la mitad de una etapa en el EDSS, o 2 puntos en una de las clasificaciones del sistema funcional apropiado (FSS), o 1 punto en dos o más de

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los FSS apropiados. El cambio se debe verificar por el investigador que examina y debe efectuar las escalas FS seleccionadas (es decir, piramidal, marcha, cerebelar, tallo cerebral, sensorial, o visual). Los síntomas deben persistir durante ≥ 24 horas y no se deben atribuir a factores clínicos que confundan (por ejemplo, fiebre, infección, lesiones, reacciones adversas a medicamentos concomitantes). Un episodio simple de un síntoma paroxismal (por ejemplo, espasmo tónico) no es una recaída, pero el nuevo ataque de ocurrencias múltiples de un síntoma paroxismal durante al menos 24 horas puede ser una recaída si se acompaña por nuevas manifestaciones, que corresponden a manifestaciones objetivas. Síntomas sensoriales que no cambian en el examen clínico, fatiga, cambio de humor, o, urgencia o incontinencia de la vejiga o el intestino no será suficiente para establecer una recaída.

La medida del resultado de la eficacia primaria es el punto de extremo MRI de lesiones que mejoran con gadolinio, o el tiempo para progresión de enfermedad confirmada (definida como un incremento de ≥ 1.0 puntos a partir de la Escala ampliada para Determinar el Estado de Incapacidad (EDSS); Kurtzke J. *Neurologi* 33(11): 1444-52 (1983)). El punto de extremo de eficacia primaria puede ser el número total de lesiones T1 mejoradas por gadolinio observadas en exploraciones MRI seriales del cerebro en las semanas 12, 16, 20, y 24.

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Medidas de resultado de eficacia secundarias incluyen frecuencia de recaída; cambio de línea base a la semana 96 en el volumen total de lesiones T2 en exploración MRI de cerebro (por ejemplo cambio en el volumen total de lesiones T2 en exploraciones MRA del cerebro a partir de selección en las semanas 24 y 36); cambio de la línea base en la semana 96 en volumen de cerebro en exploración MRI de cerebro; Escala Compuesta Funcional sobre Esclerosis Múltiple (MSFCS) y sus subescalas; función de extremidad superior, como se mide por el Ensayo Peg de 9-Agujeros (una subescala del MSFCS); ambulación, como se mide por el tiempo de camino de 25 pies (una subescala del MSFCS); cognición, según se mide por la prueba de la adición auditiva consecutiva ritmada (una subescala del MSFCS); cuestionario de calidad de vida de esclerosis múltiple 54 (MSQOL-54); volumen total de lesiones T1 de cerebro o exploraciones MRI (por ejemplo número total de lesiones T1 que mejoran con gadolinio observadas en exploraciones MRA seriales de cerebros en las semanas 20, 28, y 36); área de sección cruzada de la medula espinal cervical y en exploraciones MRI; proporción de sujetos que rehacen en las semanas 24 (es decir entre las semana 0 y la semana 24) y 36 (es decir entre la semana 0 y la semana 36); la Medición de la Actividad Única Combinada en la semana 24 y 36.

El paciente tratado con Rituximab como se describió anteriormente exhibirá una mejora en una cualquiera o más de las mediciones resultantes anteriores.

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EJEMPLO 3

TRATAMIENTO DE ESCLEROSIS MÚLTIPLE RECIDIVANTE-REMISIÓN

Un sujeto con RRMS como lo define McDonald *et al. Ann Neurol* 50:121-7 (2001) se trata con un anticuerpo CD20 aquí. En este ejemplo, la exposición al anticuerpo es de aproximadamente 1 año.

Rituximab, disponible comercialmente de Genentech, se formula para administración IV como un producto estéril en 9.0 mg/mL de cloruro de sodio, 0.7 mg/mL de polisorbato 80, 7.35 mg/mL de citrato de sodio deshidratado, y Agua Estéril para Inyección (pH 6.5).

El primer curso de tratamiento consistirá de una dosis de 1 g de Rituximab (IV) intravenoso administrada en cada uno de los días 1 y 15. Los sujetos recibirán acetaminofen (1 g) y difenhidramina HCl (50 mg), o equivalente, por la boca 30-60 minutos antes del inicio de cada infusión.

Cursos posteriores de tratamiento se administraran partiendo de la semana 48, y semana 96. La segunda infusión del curso posterior de tratamiento será de 14 ± 1 días después de la primera infusión.

El Rituximab preferiblemente es el único agente administrado para tratar la RRMS. Sin embargo, los sujetos pueden opcionalmente recibir IV o corticosteroides orales, IFN-beta,

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acetato de glatirámero, metotrexato, ciclofosfamida, o mitoxantrona.

Sujetos quienes experimentan una primera recaída puede recibir tratamiento de rescate con IV o corticosteroides orales. Corticosteroides sistémicos se pueden administrar utilizando un régimen que no excede la exposición o duración del tratamiento apropiado para una recaída MS. Una recaída en este ejemplo se define como:

La aparición de nuevos síntomas o síntomas neurológicos recurrentes consistentes con el MS durante más de 48 horas en un sujeto que a estado en un estado neurológico de mejoramiento o relativamente estable durante al menos 30 días. El cambio en síntomas neurológicos debe estar acompañado por empeoramiento neurológico consistente con un incremento de al menos la mitad de una etapa en el EDSS, o 2 porciones en una de las clasificaciones del sistema funcional apropiado (FSS), o 1 punto en dos o más de los FSS apropiados. El cambio se debe verificar mediante examen del investigador y debe afectar las escalas FS seleccionadas (es decir, piramidal, marcha, cerebelar, tallo cerebral, sensorial, o visual). Los síntomas deben persistir durante ≥ 24 horas y no se deben atribuir a factores clínicos confusos (por ejemplo, fiebre, infección, lesión, reacciones adversas a medicamentos concomitantes). Un episodio único de un síntoma paroxismal (por ejemplo, espasmo tónico) no es una recaída, pero el nuevo ataque de ocurrencias múltiples de un síntoma paroxismal durante al

menos 24 horas puede ser una recaída si se acompaña por manifestaciones objetivas nuevas, correspondientes. Síntomas sensoriales sin cambio en examen clínico, fatiga, cambio de humor, o urgencia o incontinencia de vejiga o el intestino no será suficiente para establecer una recaída.

La medida del resultado de la eficacia primaria es el punto de extremo MRI de lesiones que mejoran con gadolinio, o el tiempo para progresión de enfermedad confirmado (definid como un incremento de ≥ 1.0 puntos a partir de la Escala ampliada para Determinar el Estado de Incapacidad (EDSS); Kurtzke J. *Neurologi* 33(11): 1444-52 (1983)). El punto de extremo de eficacia primario puede ser el número total de lesiones T1 que mejoran con gadolinio observadas en exploraciones MRI seriales del cerebro en las semanas 12, 16, 20, y 24.

Medidas de resultado de eficacia secundarias incluyen frecuencia de recaída; cambio de línea base a la semana 96 en el volumen total de lesiones T2 en exploración MRI de cerebro (por ejemplo cambio en el volumen total de lesiones T2 en exploraciones MRA del cerebro a partir de exploraciones en las semanas 24 y 36); cambio de línea base en la semana 96 en volumen de cerebro en exploración MRI de cerebro; Escala Compuesta Funcional sobre Esclerosis Múltiple (MSFCS) y sus subescalas; función de extremidad superior, según se mide por el Ensayo Peg de 9-Agujeros (una subescala del MSFCS); ambulación, según se mide por el tiempo de caminar de 25 pies (una subclase del

MSFCS); cognición, según se mide por el ensayo de prueba de la adición auditiva consecutiva ritmada (una subescala del MSFCS); cuestionario de calidad de vida de esclerosis múltiple 54 (MSQOL-54); volumen total de lesiones de cerebro T1 en exploraciones MRI (por ejemplo número total de lesiones T1 mejoradas con gadolinio observadas en exploraciones MRA seriales de los cerebros en las semanas 20, 28, y 36); área de sección cruzada de la medula espina dorsal cervical y en exploraciones MRI; proporción de sujetos que recaen en la semana 24 (es decir entre las semana 0 y la semana 24) y la semana 36 (es decir entre la semana 0 y la semana 36); la Medida de la Actividad Única Combinada en la semana 24 y 36.

El paciente tratado con el anterior con Rituximab exhibirá una mejora en una cualquiera o más de las mediciones resultado anteriores.

EJEMPLO 4

VARIANTES 2H7 HUMANIZADAS

Este ejemplo describe variantes anticuerpo 2H7 humanizadas para uso en los métodos descritos aquí. El anticuerpo 2H7 humanizado comprende preferiblemente uno, dos, tres, cuatro, cinco o seis de las siguientes secuencias CDR:

Secuencia CDR L1 RASSSVSIXH en donde X es M o L (SEQ ID NO. 18), por ejemplo SEQ ID No:4 (Figura 1A),

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Secuencia CDR L2 de la SEQ ID NO:5 (Figura 1A),
Secuencia CDR L3 QQWXFNPPT en donde X es S o A (SEQ ID NO. 19), por ejemplo SEQ ID NO:6 (Figura 1A),
Secuencia CDR H1 de SEQ ID NO:10 (Figura 1B),
Secuencia CDR H2 de AIYPGNGXTSYNQKFKG en donde X es D o A (SEQ ID NO. 20), por ejemplo SEQ ID NO:11 (Figura 1B), y
Secuencia CDR H3 de VVYYSXXYWYFDV en donde X en la posición 6 es N, A, Y, W o D, y la X la posición 7 es S o R (SEQ ID NO. 21), por ejemplo SEQ ID NO: 12 (Figura 1B).

Las secuencias CDR anteriores se presentan generalmente dentro de secuencias de estructura pesadas variable y liviana variable humanas, como sustancialmente los residuos FR consenso humanos del subgrupo I (V_L6I), capa de cadena liviana humana, y sustancialmente los residuos FR consenso humanos del subgrupo III (V_HIII)de cadena pesada humana. Ver también WO 2004/056312 (Lowman *et al.*).

La región pesada variable se puede unir a una región constante de cadena IgG humana, en donde la región puede ser, por ejemplo, IgG1 o IgG3, que incluyen secuencias nativas y regiones constantes variantes.

En una modalidad preferida, tal anticuerpo comprende la secuencia de dominio pesada variable de la SEQ ID NO:8 (v16, como se muestra en la figura 1B), también opcionalmente comprende klla secuencia liviana variable de la SEQ ID NO:2 (v16, como se

muestra en la figura 1A), que comprende opcionalmente una o más sustituciones de aminoácido 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 aminoácido en las posiciones 32 y/o 92, por ejemplo M32L y/o S92A, en el dominio liviano variable. Preferiblemente, el anticuerpo es un anticuerpo intacto que comprende las secuencias de aminoácido de cadena livianas de la SEQ ID NOs. 13 o 16, y las secuencias de aminoácido de cadena pesada de la SEQ ID NO. 14, 15, 17, 22 o 25.

Un anticuerpo 2H7 humanizado preferido es ocrelizumab (Genentech). El anticuerpo aquí puede comprender adicionalmente al menos una sustitución de aminoácido en la región Fc que mejora 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, utilizando la numeración Eu de los residuos de cadena pesada. Ver también Patente estadounidense No. 6,737,056B1, Presta.

Cualquiera de estos anticuerpos pueden comprender al menos una sustitución en la región Fc que mejora la unión FcRn o vida media del suero, por ejemplo una sustitución en la posición de cadena pesada 434, tal como N434W. Ver también Patente estadounidense número 6,737,056B1, Presta. Cualquiera de estos anticuerpos pueden comprender adicionalmente al menos una sustitución de aminoácido en la región Fc que incrementa la actividad CDC, por ejemplo, que comprende al menos una

sustitución en la posición 326, preferiblemente K326A o K326W. ver también Patente estadounidense número 6,528,624B1 (Idusogie *et al.*).

Algunas variantes 2H7 humanizadas preferidas son aquellas que comprenden el dominio liviano variable de las SEQ ID NO:2 y el dominio pesado variable de la SEQ ID NO:8, que incluyen aquellas con o sin sustituciones en una región Fc (si está presente), y aquellas que comprenden un dominio pesado variable con alteraciones N100A; o D56A y N100A; o D56A, N100Y, y S100aR; en la SEQ ID NO:8 y un dominio liviano variable con alteración M32L; o S92A; o M32L y S92A; en la SEQ ID NO:2. M34 en el dominio pesado variable de 2H7.v16 se ha identificado como una fuente potencial de estabilidad de anticuerpo y es otro candidato potencial para sustitución.

En un resumen de algunas varias modalidades preferidas de la invención, la región variables de las variantes basadas en 2H7.v16 comprende las secuencias de aminoácido de v16 excepto en las posiciones de aminoácido que se indican en la tabla adelante. A menos que se indique otra cosa, las variantes 2H7 tendrán la misma cadena liviana como aquella del v16.

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Variantes de anticuerpo 2H7 humanizadas de ejemplo

Versión 2H7	Cambios (V_H) de cadena pesada	Cambios (VL) de cadena liviana	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, S100aR	M32L, S92A	S298A, E333A, K334A, K326A

Un 2H7 humanizado preferido comprende la secuencia de dominio liviana variable 2H7.vl6:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

Y la secuencia de dominio pesado variable 2H7.vl6:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SS (SEQ ID NO:8).

En donde el anticuerpo 2H7.v16 humanizado es un anticuerpo intacto, este puede comprender la secuencia de aminoácido de cadena liviana:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

Y la secuencia de aminoácido de cadena pesada de la SEQ ID NO. 14 o:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPG (SEQ ID NO:22).

Otro anticuerpo 2H7 humanizado preferido comprende la secuencia de dominio liviano variable 2H7.v511:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:23)

Y la secuencia de dominio pesado variable 2H7.v511:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGN GATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQ GTLVTVSS (SEQ ID NO. 24).

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En donde el anticuerpo 2H7.v511 humanizado es un anticuerpo intacto, este puede comprender la secuencia de aminoácido de cadena liviana:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGS
GSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:16)

Y la secuencia de aminoácido de cadena pesada de la SEQ ID NO. 17 o:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR
VVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 25).

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REIVINDICACIONES

LO QUE SE REIVINDICA ES:

1. Un método para tratar esclerosis múltiple en un sujeto que comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto para suministrar una exposición inicial a anticuerpo de aproximadamente 0.5 a 4 gramos seguido por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se suministra hasta aproximadamente 16 a 60 semanas a partir de la exposición inicial, y cada una de las exposiciones de anticuerpo se suministra al sujeto como una o dos dosis de anticuerpo.

2. El método de la reivindicación 1 en donde la segunda exposición no se suministra hasta de aproximadamente 20 a 30 semanas partir de la exposición inicial.

3. El método de la reivindicación 1 o 2 en donde la exposición inicial y la segunda exposición de anticuerpo cada una se suministran en cantidades de aproximadamente 1.5 a 2.5 gramos.

4. El método de una cualquiera de las reivindicación 2-3 que comprende adicionalmente administrar al sujeto una cantidad efectiva del anticuerpo CD20 para suministrar una tercera

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exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la tercera exposición no se administra hasta aproximadamente 46 a 60 semanas a partir de la exposición inicial.

5. El método de la reivindicación 4 en donde la tercera exposición anticuerpo se suministra en una cantidad de aproximadamente 01.5 a 2.5 gramos.

6. El método de la reivindicación 4 o 5 en donde la tercera exposición no se suministra hasta aproximadamente 46 a 54 semanas a partir de la exposición inicial.

7. El método de una cualquiera de las reivindicaciones 4-6 en donde ninguna exposición adicional a anticuerpo se suministra hasta al menos aproximadamente 70-75 semanas a partir de la exposición inicial.

8. El método de la reivindicación 1 en donde la segunda exposición no se suministra hasta aproximadamente 46 a 60 semanas a partir de la exposición inicial.

9. El método de una cualquiera de las reivindicaciones 1-8 en donde cada una de las exposiciones de anticuerpos se suministra al sujeto como una dosis del anticuerpo.

10. El método de una cualquiera de las reivindicaciones 1-8 en donde cada una de las exposiciones de anticuerpo se suministra al sujeto como dos dosis de anticuerpo, en donde las dos dosis constituyen una primer dosis y una segunda dosis.

11. El método de la reivindicación 10 en donde la según da dosis se suministra de aproximadamente 3-17 días a partir del tiempo que se administra la primera dosis.

12. El método de la reivindicación 11 en donde la segunda dosis se administra aproximadamente 6-16 días a partir del tiempo en que se administra la primer dosis.

13. El método de la reivindicación 12 en donde la segunda dosis se administra aproximadamente 13-16 días a partir del tiempo en que se administra la primer dosis.

14. El método de una cualquiera de las reivindicaciones 10-13 en donde la primera y segunda dosis de anticuerpo cada una son de aproximadamente 0.5 a 1.5 gramos.

15. El Método de una cualquiera de las reivindicaciones 10-14 en donde la primera y segunda dosis de anticuerpo cada uno son de aproximadamente 0.75 a 1.3 gramos.

16. El método de una cualquiera de la reivindicaciones 1-15 en donde tres o más exposiciones de anticuerpo se administran al sujeto.

17. El método de una cualquiera de las reivindicaciones 1-16 en donde cuatro o más exposiciones de anticuerpo se administran al sujeto.

18. El método de una cualquiera de las reivindicaciones 1-17 en donde un segundo medicamento se administra con la exposición inicial a exposiciones posteriores, en donde el anticuerpo CD20 es un primer medicamento.

19. El método de la reivindicación 18 en donde el segundo medicamento se selecciona del grupo que consiste de un interferón, acetato glatirámero, un agente citotóxico, agente quimioterapéutico, mitoxantrona, metotrexato, ciclofosfamida, clorambucil, azatioprina, gamma globulina, Campath, anti-CD4, cladribina, corticosteroide, mofetil micofenolate (MMF), ciclosporina, droga reductora del colesterol de la clase estatina, estradiol, testosterona, droga de reemplazo de hormona, un inhibidor TNF, droga antirreumática que modifica la enfermedad (DMARD), droga antiinflamatoria no-esteroide (NSAID), levotiroxina, ciclosporina A, análogo de somatastatina, citoquina o antagonista del receptor citoquina, antimetabolito, agente inmunosupresor, antagonista de integrina o anticuerpo,

anticuerpo LFA-I, efalizumab, anticuerpo de integrina alfa 4, natalizumab, y otro anticuerpo marcadores se superficie de célula B.

20. El método de una cualquiera de las reivindicaciones 1-19 en donde la esclerosis múltiple es esclerosis múltiple recidivante-remitente (RRMS).

21. El método de una cualquiera de las reivindicaciones 1-19 en donde la esclerosis múltiple es esclerosis múltiple progresiva primaria (PPMS).

22. El método de una cualquiera de las reivindicaciones 1-21 en donde el sujeto nunca ha sido tratado previamente con un anticuerpo CD20.

23. El método de una cualquiera de las reivindicaciones 1-22 en donde el anticuerpo es un anticuerpo desnudo.

24. El método de una cualquiera de las reivindicaciones 1-22 en donde el anticuerpo se conjuga con otra molécula.

25. El método de la reivindicación 24 en donde la otra molécula es un agente citotóxico.

26. El método de una cualquiera de las reivindicaciones 1-25 en donde del anticuerpo se administra intravenosamente.

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27. El método de la reivindicación 26 en donde el anticuerpo se administra intravenosamente para cada exposición de anticuerpo.

28. El método de una cualquiera de las reivindicaciones 1-25 en donde el anticuerpo se administra subcutáneamente.

29. El método de una cualquiera de las reivindicaciones 1-25 en donde el anticuerpo se administra intratecálmente.

30. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo CD20 es el único medicamento administrado al sujeto para tratar la esclerosis múltiple.

31. El método de una cualquiera de las reivindicaciones 1-30 en donde el anticuerpo es Rituximab.

32. El método de una cualquiera de las reivindicaciones 1-30 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en la SEQ ID NOS. 2 y 8.

33. El método de una cualquiera de las reivindicaciones 1-30 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en la SEQ ID NOS. 23 y 24.

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34. El método de una cualquiera de las reivindicaciones 1-34 en donde el sujeto tiene proteína básica antimielina elevada (MBP), glucoproteína oligodendrocitaria anti-mielina (MOG), niveles de anticuerpo anti-neurofilamento y/o anti-gangliosida.

35. El método de una cualquiera de las reivindicaciones 1-34 en donde niveles elevados de células B están presentes en fluidos cerebroespinal (CSF), lesión de esclerosis múltiple, o suero del sujeto.

36. Un artículo de fabricación que comprende:

(a) un contenedor que contiene un anticuerpo CD20; y

(b) un inserto de paquete con instrucciones para tratar esclerosis múltiple en un sujeto, en donde las instrucciones indican que una cantidad del anticuerpo se administra al sujeto que es efectiva para suministrar una exposición al anticuerpo inicial de aproximadamente 0.5 a 4 gramos seguido por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se administra hasta aproximadamente 16 a 60 semanas de exposición inicial y cada una de las exposiciones de anticuerpo se suministra al sujeto como una o dos dosis de anticuerpo.

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37. El artículo de la reivindicación 36 que comprende adicionalmente un contenedor que comprende un segundo medicamento, en donde el anticuerpo CD20 es un primer medicamento, y comprende adicionalmente instrucciones en el inserto de paquete para tratar el sujeto con un segundo medicamento.

38. El artículo de la reivindicación 37 en donde el segundo medicamento se selecciona del grupo que consiste de un interferón, acetato glatirámero, un agente citotóxico, agente quimioterapéutico, mitoxantrona, metotrexato, ciclofosfamida, clorambucil, azatioprina, gamma globulina, Campath, anti-CD4, cladribina, corticosteroide, mofetil micofenolato (MMF), ciclosporina, droga reductora de colesterol de la clase estatina, estradiol, testosterona, droga de reemplazo de hormona, un inhibidor TNF, droga antirreumática que modifica la enfermedad (DMARD), droga antiinflamatoria no-esteroide (NSAID), levotiroxina, ciclosporina A, análogo de somatastatina, citoquina o antagonista del receptor citoquina, antimetabolito, agente inmunosupresor, y otro anticuerpo marcador de superficie célula B.

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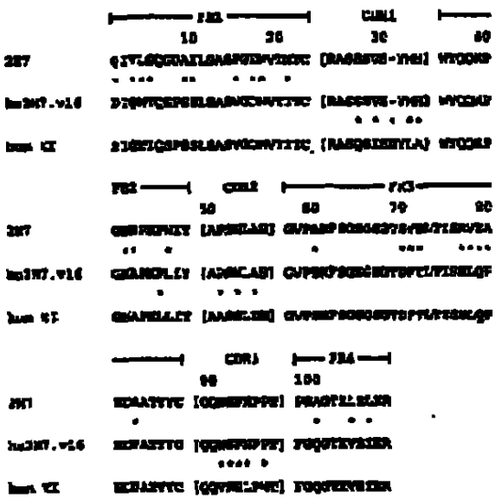
MÉTODO PARA TRATAR ESCLEROSIS MÚLTIPLE

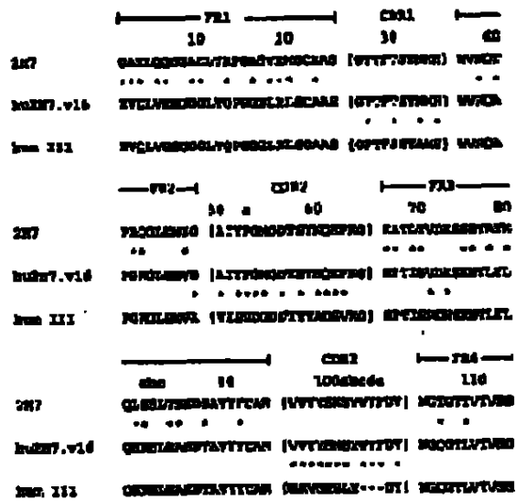
RESUMEN

Se describen métodos para tratar esclerosis múltiple (MS) con un anticuerpo CD20 que utiliza regímenes y protocolos de dosificación especial. También se describen artículos de elaboración para uso en tales métodos.

Secuencia de Alineación de Dominio de Cadena Liviana Variable



Secuencia de Alineación de Dominio de Cadena Pesada Variable



Secuencia de Alineación de Dominio de Cadena Pesada Variable

Cadena Liviana de 2H7.v16 Humanizado

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG._2

Cadena Pesada de 2H7.v16 Humanizado

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

FIG._3

Cadena Pesada de 2H7.v31 Humanizado

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

FIG._4

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Alineación de Cadena Liviana

```
                1                                32
hu2H7.v16       DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHNYQQKPGKAPKPLIYAP
                ********************************.*******************
hu2H7.v511      DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHMYQQKPGKAPKPLIYAP

               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._5

Alineación de Cadena Pesada

```
                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
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FIG._6

102.493

Listado de Secuencia

<110> GENENTECH, INC.
FROHNA, PAUL A.

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<130> P2134R1 PCT

<140> PCT/US2005/019641
<141> 2005-06-02

<150> US 60/576,993
<151> 2004-06-04

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				20				25						30	
Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser	Pro	Lys	Pro	
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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	
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Lys	Arg														

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<223> Se sintetiza secuencia.

<400> 2

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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														

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<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 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

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<212> PRT
<213> Mus musculus

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5 10

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<212> PRT
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Ala Pro Ser Asn Leu Ala Ser
5

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<211> 122
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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

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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

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20 25 30

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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 Thr 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

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Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
5 10

<210> 11
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1 5 10 15

Lys Gly

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<213> Mus musculus

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5 10

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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

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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
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Gly Lys

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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 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 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

Lys 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> 16
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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
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Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
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Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
200 205 210

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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 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

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<210> 19
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<222> 4
<223> Xaa es S o A

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<210> 20
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<220>
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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 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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<210> 23
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Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
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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

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

Lys Arg

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

<210> 25
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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 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

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W4/5

L

PATENT COOPERATION TREA[illegible]

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

MAY 0 8 2006
GENENTECH, INC.
LEGAL DEPT

Applicant's or agent's file reference P2134R1	Date of mailing (day/month/year) 03/05/2006
International application No. PCT/US2005/019641	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant GENENTECH, INC.	International filing date (day/month/year) 02/06/2005

Calendared/LRS
Response to ISR deadline / Consider
Chap II 3 JUL 06

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

IDS-PCT search
7 JUN 06
(P2134R1 US)

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 90bis.1 and 90bis.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 20 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

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P2134R1	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2005/019641	International filing date (day/month/year) 02/06/2005	(Earliest) Priority Date (day/month/year) 04/06/2004
Applicant GENENTECH, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 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,
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. 1
      [X] 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. [ ] none of the figures is to be published with the abstract

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/019641

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:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/019641

A. CLASSIFICATION OF SUBJECT MATTER
A61K39/395 A61P25/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C07K

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, EMBASE, BIOSIS, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/035607 A (GENMAB A/S; MEDAREX, INC; TEELING, JESSICA; RUULS, SIGRID; GLENNIE, MA) 29 April 2004 (2004-04-29) page 59, line 30 page 53, line 20 - line 36	1-38
X	WO 02/22212 A (IDEC PHARMACEUTICALS CORPORATION) 21 March 2002 (2002-03-21) claim 30	18-35, 37,38
X	WO 01/13945 A (BIOCRYSTAL LTD) 1 March 2001 (2001-03-01) example 4	1-38
X	WO 03/068821 A (IMMUNOMEDICS, INC; MCCALL, JOHN, DOUGLAS) 21 August 2003 (2003-08-21)	36-38
Y	the whole document	1-38

-/—

[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
24 February 2006	03/05/2006

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	Perez, F

Form PCT/ISA/210 (second sheet) (April 2005)

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International application No
PCT/US2005/019641

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COLES A J ET AL: "Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 354, no. 9191, 13 November 1999 (1999-11-13), pages 1691-1695, XP004831069 ISSN: 0140-6736 the whole document	1-38
Y	COLES A J ET AL: "MONOCLONAL ANTIBODY TREATMENT EXPOSES THREE MECHANISMS UNDERLYING THE CLINICAL COURSE OF MULTIPLE SCLEROSIS" ANNALS OF NEUROLOGY, BOSTON, US, vol. 46, no. 3, September 1999 (1999-09), pages 296-304, XP008017899 ISSN: 0364-5134 the whole document	1-38
Y	US 2004/072749 A1 (ZOCHOER MARCEL ET AL) 15 April 2004 (2004-04-15) the whole document	1-38

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/019641

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.: 1-35
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-35 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.

information on patent family members

International application No
PCT/US2005/019641

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004035607 A	29-04-2004	AU 2003301445 A1	04-05-2004
		BR 0315295 A	30-08-2005
		CA 2502552 A1	29-04-2004
		EP 1558648 A2	03-08-2005
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PATENT COOPERATION TREATY

From the:
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/019641	02.06.2005	04.06.2004

International Patent Classification (IPC) or both national classification and IPC
INV. A61K39/395 A61P25/00

Applicant
GENENTECH, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV *Lack of unity of invention*
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
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Authorized Officer

Perez, F

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/019641

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/019641

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. 1-35

because:

☒ the said international application, or the said claims Nos. 1-35 (industrial applicability) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/019641

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-35
	No:	Claims	36-38
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-38
Industrial applicability (IA)	Yes:	Claims	36-38
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2005/019641

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 1-35 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 under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 2004/035607 A (GENMAB A/S; MEDAREX, INC; TEELING, JESSICA; RUULS, SIGRID; GLENNIE, MA) 29 April 2004
D2: WO 02/22212 A (IDEC PHARMACEUTICALS CORPORATION) 21 March 2002
D3: WO 01/13945 A (BIOCRYSTAL LTD) 1 March 2001
D4: WO 03/068821 A (IMMUNOMEDICS, INC; MCCALL, JOHN, DOUGLAS) 21 August 2003
D5: COLES A J ET AL: "Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis" LANCET THE, LANCET LIMITED. LONDON, GB, vol. 354, no. 9191, 13 November 1999 (1999-11-13), pages 1691-1695
D6: COLES A J ET AL: "MONOCLONAL ANTIBODY TREATMENT EXPOSES THREE MECHANISMS UNDERLYING THE CLINICAL COURSE OF MULTIPLE SCLEROSIS" ANNALS OF NEUROLOGY, BOSTON, US, vol. 46, no. 3, September 1999 (1999-09), pages 296-304
D7: US 2004/072749 A1 (ZOCHOER MARCEL ET AL) 15 April 2004

Novelty (Articles 33.1 and 33.2 PCT)

Claim 1 relates to a method of treating multiple sclerosis comprising administering CD20 antibody in two exposures separated by 16-60 weeks.

Whereas D1-D2 disclose the use of CD20 antibody for treating multiple sclerosis, none disclose the dosage regimen covered by claim 1. Claims 1-35 are therefore novel.

Claims 36-38 cover articles of manufactures. Instructions of use are not considered as a technical feature susceptible to differentiate a compound. The articles of manufactures are therefore characterised as a container comprising a CD20 antibody, and optionally a second container containing a second medicament.

CD20 antibody is a well known product in the prior art which thus is packaged into a container and CD20 antibodies are commonly associated with other medicaments. Claims 36-38 therefore lack novelty.

Inventive Step (Articles 33.1 and 33.3 PCT)

The document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and discloses the use of CD20 antibody for treating multiple sclerosis using a single exposure of the antibody.

The subject-matter of claim 1 therefore differs from this known treatment in that a second injection is performed 16-50 week after the first injection.

The problem to be solved by the present invention may therefore be regarded as providing a different dosage regimen for the treatment of multiple sclerosis with CD20 antibody.

The application nevertheless fails to show any technical advantage which is new and surprising, and that could convincingly be related to a second administration. The application can therefore be construed as an arbitrary modification of the treatments of multiple sclerosis known from the prior art.

Consequently, the present application cannot be considered as involving an inventive step (Article 33(3) PCT).

Industrial applicability (Articles 33.1 and 33.4 PCT)

For the assessment of the present claims 1-35 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Further remarks

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D6 is not mentioned in the description, nor are these documents identified therein.

It should be noted that upon entry within the regional phase, the allowability of claim 1 would be reconsidered in the light of Article 52(4) EPC.

PATENT COOPERATION TREATY

PCT/US2005/019641

SEP 0 7 2005

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)* 25 August 2005 (25.08.2005)	
Applicant's or agent's file reference P2134R1	IMPORTANT NOTIFICATION
International application No. PCT/US2005/019641	International filing date *(day/month/year)* 02 June 2005 (02.06.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 04 June 2004 (04.06.2004)
Applicant GENENTECH, INC. et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)* An asterisk (*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
04 June 2004 (04.06.2004)	60/576,893	US	15 July 2005 (15.07.2005)

The International Bureau of WIPO
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PCT REQUEST

Original (for SUBMISSION)

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form PCT/RO/101 PCT Request	
0-4-1	Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.170)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	Applicant's or agent's file reference	P2134R1
I	Title of invention	METHOD FOR TREATING MULTIPLE SCLEROSIS
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)	FROEHNA, Paul A.
III-1-5	Address	3880 19th Street San Francisco, California 94114 United States of America
III-1-6	State of nationality	US
III-1-7	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.	650-225-1000
IV-1-4	Facsimile No.	650-952-9881
IV-1-6	Agent's registration No.	40,378
IV-2	Additional agent(s)	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	AGARWAL,Atulya R.; ANDRUS,Alex; BARNES,Elizabeth M.; CARPENTER,David A.; COBURN,Cara M.; CONLEY,Deirdre L.; CUI,Steven X.; EVANS,David W.; HASAK,Janet E.; JOHNSTON,Sean A.; KALINOWSKI,Grant; KRESHAK,Mark T.; KUBINEC,Jeffrey S.; MARSCHANG,Diane L.; NAIK,Paul; PLEASURE,Irene; SCHWARTZ,Timothy R.; SHERMAN,Daniel E.; 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	04 June 2004 (04.06.2004)
VI-1-2	Number	60/576,993
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

P2134R1

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PCT REQUEST

Original (for SUBMISSION)

VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	4	✓
IX-2	Description	55	-
IX-3	Claims	3	-
IX-4	Abstract	1	✓
IX-5	Drawings	5	-
IX-7	TOTAL	68	
	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-18	Figure of the drawings which should accompany the abstract	1	
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative	WM Lee	
X-1-1	Name (LAST, First)	LEE, Wendy M.	
X-1-2	Name of signatory		
X-1-3	Capacity		

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau:	

METHOD FOR TREATING MULTIPLE SCLEROSIS

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Field of the Invention

The present invention concerns methods for treating multiple sclerosis (MS) in a subject using special dosing regimens and protocols, and an article of manufacture with instructions for such use.

Background of the Invention

Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory and demyelinating degenerative disease of the human central nervous system (CNS). It is a worldwide disease that affects approximately 300,000 persons in the United States; it is a disease of young adults, with 70%–80% having onset between 20 and 40 years old (Anderson *et al. Ann Neurology* 31(3):333–6 (1992); Noonan *et al. Neurology* 58:136–8 (2002)). MS is a heterogeneous disorder based on clinical course, magnetic resonance imaging (MRI) scan assessment, and pathology analysis of biopsy and autopsy material (Lucchinetti *et al. Ann Neurol* 47:707–17 (2000)). The disease manifests itself in a large number of possible combinations of deficits, including spinal cord, brainstem, cranial nerve, cerebellar, cerebral, and cognitive syndromes. Progressive disability is the fate of most patients with MS, especially when a 25-year perspective is included. Half of MS patients require a cane to walk within 15 years of disease onset. MS is a major cause of neurologic disability in young and middle-aged adults and, until the past decade, has had no known beneficial treatments. MS is difficult to diagnose because of the non-specific clinical findings, which led to the development of highly structured diagnostic criteria that include several technological advances, consisting of MRI scans, evoked potentials, and cerebrospinal fluid (CSF) studies. All diagnostic criteria rely upon the general principles of scattered lesions in the central white matter occurring at different times and not explained by other etiologies such as infection, vascular disorder, or autoimmune disorder (McDonald *et al. Ann Neurol* 50:121–7 (2001)). MS has four patterns of disease: relapsing-remitting MS (RRMS; 80%–85% of cases at onset), primary progressive MS (PPMS; 10%–15% at onset), progressive relapsing MS (PRMS; 5% at onset); and secondary progressive MS (SPMS) (Kremenchutzky *et al. Brain* 122 (Pt 10):1941–50 (1999); Confavreux *et al. N Engl J Med* 343(20):1430–8 (2000)). An estimated 50% of patients with RRMS will develop SPMS in 10 years, and up to 90% of RRMS patients will eventually develop SPMS (Weinshenker *et al. Brain* 112(Pt 1):133–46 (1989)).

Currently, six drugs in four classes are approved in the United States for the treatment of RRMS, whereas no drugs have been approved for PPMS. The RRMS treatments include the following: interferon class, IFN-beta-1a (REBIF® and AVONEX®) and IFN-beta-1b (BETASERON®); glatiramer acetate (COPAXONE®), a polypeptide; natalizumab (TYSABRI®); and mitoxantrone (NOVANTRONE®), a cytotoxic agent. Other drugs have been used with varying

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degrees of success, including corticosteroids, methotrexate, cyclophosphamide, azathioprine, and intravenous (IV) immunoglobulin. The benefits of currently approved treatments are relatively modest (~30%) for relapse rate and prevention of disability in RRMS as suggested by two recent meta-analyses (Filippini *et al. Lancet* 361:545–52 (2003)).

Other clinical studies evaluated other immunomodulatory agents in MS, including tumor necrosis factor–α inhibitors and altered peptide ligands, which aggravated rather than improved MS (Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI *Neurology* 53:457–65 (1999); Bielekova *et al. Nat Med* 2000;6:1167–75 (2000), erratum appears in *Nat Med* 6:1412 (2000)).

The predominant view of MS pathophysiology has held that inflammation is principally mediated by $CD4^+$ Th1 T cells. Therapeutic approaches based on this theory such as IFN-beta and glatiramer acetate decrease, but do not fully prevent, occurrence of exacerbations or accumulation of disability.

The existence of a humoral component in human MS has been implicitly recognized for decades, as evidenced by inclusion of CSF oligoclonal bands and increased intrathecal IgG synthesis in diagnostic criteria for MS (Siden A. *J Neurol* 221:39–51(1979); McDonald *et al. Ann Neurol* 50:121–7 (2001); Andersson *et al. Eur J Neurol* 9:243–51 (2002); O'Connor, P. *Neurology* 59:S1–33 (2002)). The presence of oligoclonal bands, increased free light chains, and increased intrathecal IgM synthesis correlates with MS disease activity and may be a predictor of more severe outcomes (Rudick *et al. Mult Scler* 1:150–5 (1995); Zeman *et al. Acta Cytol* 45:51–9 (2001); Izquierdo *et al. Acta Neurol Scand* 105:158–63 (2002); Wolinsky J. *J Neurol Sci* 206:145–52 (2003); Villar *et al. Ann Neurol* 53:222–6 (2003)).

Anti-myelin antibodies (myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG)) have been detected in the serum of patients with progressive and relapsing forms of MS (Reindl *et al. Brain* 122:2047–56 (1999); Egg *et al. Mult Scler* 7(5):285–9 (2001)). Anti-myelin antibodies have also been detected in the CSF of MS patients (Reindl *et al. Brain* 122:2047–56 (1999); Egg *et al. Mult Scler* 7(5):285–9 (2001); Andersson *et al. Eur J Neurol* 9:243–51 (2002)). Additional types of antibodies such as anti-ganglioside antibodies or anti-neurofilament antibodies have been observed in patients with MS (Mata *et al. Mult Scler* 5:379–88 (1999); Sadatipour *et al. Ann Neurol* 44:980–3 (1998)). A recent report indicated that the presence of serum anti-MOG and anti-MBP antibodies was a strong predictor of progression from a clinically isolated demyelinating event to definite RRMS (Berger *et al. N Engl J Med* 349:139–45 (2003)). The adjusted hazard ratio for experiencing an exacerbation was 76.5 for patients who were seropositive for both antibodies and 31.6 for patients who were seropositive only for anti-MOG.

An international pathology consortium found that antibodies bound to myelin are present in the majority of patients with MS, with plasma cells and B cells also found in MS lesions, providing

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additional evidence for a humoral role in MS (Prineas and Wright, *Lab Invest* 38:409–21 (1978); Esiri M. *Neuropathol Appl Neurobiol* 6:9–21 (1980); Genain *et al. Nat Med* 5:170–5 (1999); Lucchinetti *et al. Ann Neurol* 47:707–17 (2000); Wingerchuk *et al. Lab Invest* 81:263–81 (2001)). B cells are detectable in the CSF of patients with MS, and the presence of a relatively high proportion of B cells may be predictive of more severe disability progression (Cepok *et al. Brain* 124(Pt 11):2169–76 (2001)).

In subjects with RRMS or opsoclonus-myoclonus syndrome, Rituximab reportedly depleted peripheral B cells in all subjects and decreased the number of CSF B cells in some patients (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003); Cross *et al.* "Preliminary Results from a Phase II Trial of Rituximab in MS" (abstract) *Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis ACTRIMS* 20–1 (October, 2003)). See also Cree *et al.* "Tolerability and Effects of Rituximab "Anti-CD20 Antibody" in Neuromyelitis Optica and Rapidly Worsening Multiple Sclerosis" *Meeting of the Am. Acad. Neurol.* (April, 2004).

CD20 Antibodies and Therapy Therewith

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

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

The CD20 antigen (also called human B-lymphocyte-restricted differentiation antigen, Bp35) is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes (Valentine *et al. J. Biol. Chem.* 264(19):11282-11287 (1989); and Einfeld *et al. EMBO J.* 7(3):711-717 (1988)). The antigen is also expressed on greater than 90% of B-cell non-Hodgkin's lymphomas (NHL) (Anderson *et al. Blood* 63(6):1424-1433 (1984)), but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues (Tedder *et al. J. Immunol.* 135(2):973-979 (1985)). CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation (Tedder *et al., supra*) and possibly functions as a calcium ion channel (Tedder *et al. J. Cell. Biochem.* 14D:195 (1990)).

Given the expression of CD20 in B-cell lymphomas, this antigen can serve as a candidate for "targeting" of such lymphomas. In essence, such targeting can be generalized as follows: antibodies specific to the CD20 surface antigen of B cells are administered to a patient. These anti-CD20

antibodies specifically bind to the CD20 antigen of (ostensibly) both normal and malignant B cells; the antibody bound to the CD20 surface antigen may lead to the destruction and depletion of neoplastic B cells. Additionally, chemical agents or radioactive labels having the potential to destroy the tumor can be conjugated to the anti-CD20 antibody such that the agent is specifically "delivered" to the neoplastic B cells. Irrespective of the approach, a primary goal is to destroy the tumor; the specific approach can be determined by the particular anti-CD20 antibody that is utilized and, thus, the available approaches to targeting the CD20 antigen can vary considerably.

The Rituximab (RITUXAN®) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen. Rituximab is the antibody called "C2B8" in US Patent No. 5,736,137 issued April 7, 1998 (Anderson *et al.*). RITUXAN® is indicated for the treatment of patients with relapsed or refractory low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma. *In vitro* mechanism of action studies have demonstrated that RITUXAN® binds human complement and lyses lymphoid B-cell lines through complement-dependent cytotoxicity (CDC) (Reff *et al. Blood* 83(2):435-445 (1994)). Additionally, it has significant activity in assays for antibody-dependent cellular cytotoxicity (ADCC). More recently, RITUXAN® has been shown to have anti-proliferative effects in tritiated thymidine incorporation assays and to induce apoptosis directly, while other anti-CD19 and CD20 antibodies do not (Maloney *et al. Blood* 88(10):637a (1996)). Synergy between RITUXAN® and chemotherapies and toxins has also been observed experimentally. In particular, RITUXAN® sensitizes drug-resistant human B-cell lymphoma cell lines to the cytotoxic effects of doxorubicin, CDDP, VP-16, diphtheria toxin and ricin (Demidem *et al. Cancer Chemotherapy & Radiopharmaceuticals* 12(3):177-186 (1997)). *In vivo* preclinical studies have shown that RITUXAN® depletes B cells from the peripheral blood, lymph nodes, and bone marrow of cynomolgus monkeys, presumably through complement and cell-mediated processes (Reff *et al. Blood* 83(2):435-445 (1994)).

Rituximab was approved in the United States in November 1997 for the treatment of patients with relapsed or refractory low-grade or follicular $CD20^+$ B-cell non-Hodgkin's lymphoma (NHL) at a dose of 375 mg/m^2 weekly for four doses. In April 2001, the Food and Drug Administration (FDA) approved additional claims for the treatment of low-grade NHL: retreatment (weekly for four doses) and an additional dosing regimen (weekly for eight doses). There have been more than 300,000 patient exposures to Rituximab either as monotherapy or in combination with immunosuppressant or chemotherapeutic drugs. Patients have also been treated with Rituximab as maintenance therapy for up to 2 years (Hainsworth *et al. J Clin Oncol* 21:1746–51 (2003); Hainsworth *et al. J Clin Oncol* 20:4261–7 (2002)).

Rituximab has also been studied in a variety of non-malignant autoimmune disorders, in which B cells and autoantibodies appear to play a role in disease pathophysiology (Edwards *et al. Biochem Soc Trans* 30:824–8 (2002)). Rituximab has been reported to potentially relieve signs and symptoms of rheumatoid arthritis (RA) (Leandro *et al. Ann Rheum Dis.* 61:883–8 (2002); Emery *et*

al. Arthritis Rheum 48(9):S439 (2003)), lupus (Eisenberg R. *Arthritis Res Ther* 5:157–9 (2003); Leandro *et al. Arthritis Rheum* 46:2673–7 (2002)), immune thrombocytopenia (D'Arena *et al. Leuk Lymphoma* 44:561–2 (2003)), autoimmune anemia (Zaja *et al. Haematologica* 87:189–95 (2002) (erratum appears in *Haematologica* 87:336 (2002)), autoimmune neuropathy (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485–9 (2003)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), and relapsing-remitting multiple sclerosis (RRMS) (Cross *et al.* (abstract) Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis 20–1 (2003)).

A Phase II study (WA16291) has been conducted in patients with rheumatoid arthritis (RA), providing 48-week follow-up data on safety and efficacy of Rituximab (Emery *et al. Arthritis Rheum* 48(9):S439 (2003); Szczepanski *et al. Arthritis Rheum* 48(9):S121 (2003)). A total of 161 patients were evenly randomized to four treatment arms: methotrexate, Rituximab alone, Rituximab plus methotrexate, Rituximab plus cyclophosphamide (CTX). The treatment regimen of Rituximab was 1 g administered intravenously on Days 1 and 15. Infusions of Rituximab in most patients with RA were well tolerated by most patients, with 36% of patients experiencing at least one adverse event during their first infusion (compared with 30% of patients receiving placebo). Overall, the majority of adverse events were considered to be mild to moderate in severity and were well balanced across all treatment groups. There were a total of 19 serious adverse events across the four arms over the 48 weeks, which were slightly more frequent in the Rituximab/CTX group. The incidence of infections was well balanced across all groups. The mean rate of serious infection in this RA patient population was 4.6 6 per 100 patient-years, which is lower than the rate of infections requiring hospital admission in RA patients (9.57 per 100 patient-years) reported in a community-based epidemiologic study (Doran *et al. Arthritis Rheum* 46:2287–93 (2002)).

The reported safety profile of Rituximab in a small number of patients with neurologic disorders, including autoimmune neuropathy (Pestronk *et al. J Neurol Neurosurg Psychiatry* 74:485–9 (2003)), opsoclonus/myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl1) PO5.128:A395 (2003)), and RRMS (Cross *et al.* Preliminary results from a phase II trial of Rituximab in MS (abstract) Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis 20–1 (2003)), was similar to that reported in oncology or RA. In an ongoing investigator-sponsored trial (IST) of Rituximab in combination with interferon-beta (IFN-beta) or glatiramer acetate in subjects with RRMS (Cross *et al.*, *supra*), 1 of 10 treated subjects was admitted to the hospital for overnight observation after experiencing moderate fever and rigors following the first infusion of Rituximab, while the other 9 subjects completed the four-infusion regimen without any reported adverse events.

Patents and patent publications concerning CD20 antibodies include US Patent Nos. 5,776,456, 5,736,137, 5,843,439, 6,399,061, and 6,682,734, as well as US 2002/0197255, US 2003/0021781, US 2003/0082172, US 2003/0095963, US 2003/0147885 (Anderson *et al.)*; US Patent

No. 6,455,043, US 2003/0026804, and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); WO 2000/27433 and US 2004/0213784 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO01/10461 (Rastetter and White); WO 2001/10460 (White and Grillo-Lopez); US 2001/0018041, US 2003/0180292, WO 2001/34194 (Hanna and Hariharan); US 2002/0006404 and WO 2002/04021 (Hanna and Hariharan); US 2002/0012665 and WO 2001/74388 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858 (White, C.); US 2002/0128488 and WO 2002/34790 (Reff, M.); WO 2002/060955 (Braslawsky *et al.*);WO 2002/096948 (Braslawsky *et al.*);WO 2002/079255 (Reff and Davies); US Patent No. 6,171,586 and WO 1998/56418 (Lam *et al.*); WO 1998/58964 (Raju, S.); WO 1999/22764 (Raju, S.); WO 1999/51642, US Patent No. 6,194,551, US Patent No. 6,242,195, US Patent No. 6,528,624 and US Patent No. 6,538,124 (Idusogie *et al.*); WO 2000/42072 (Presta, L.); WO 2000/67796 (Curd *et al.*); WO 2001/03734 (Grillo-Lopez *et al.*); US 2002/0004587 and WO 2001/77342 (Miller and Presta); US 2002/0197256 (Grewal, I.); US 2003/0157108 (Presta, L.); WO 04/056312 (Lowman *et al.*); US 2004/0202658 and WO 2004/091657 (Benyunes, K.); WO 2005/000351 (Chan, A.); US 2005/0032130A1 (Beresini *et al.*); US 2005/0053602A1 (Brunetta, P.); US Patent Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721, (Kaminski *et al.*); US Patent Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, and 6,652,852 (Robinson *et al.*); US Pat No. 6,410,391 (Raubitschek *et al.)*; US Patent No. 6,224,866 and WO00/20864 (Barbera-Guillem, E.); WO 2001/13945 (Barbera-Guillem, E.); US2005/0079174A1 (Barbera-Guillem *et al.*); WO 2000/67795 (Goldenberg); US 2003/0133930 and WO 2000/74718 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO2004/058298 (Goldenberg and Hansen); WO 2000/76542 (Golay *et al.*);WO 2001/72333 (Wolin and Rosenblatt); US Patent No. 6,368,596 (Ghetie *et al.*); US Patent No. 6,306,393 and US 2002/0041847 (Goldenberg, D.); US 2003/0026801 (Weiner and Hartmann); WO 2002/102312 (Engleman, E.); US 2003/0068664 (Albitar *et al.*); WO 2003/002607 (Leung, S.); WO 2003/049694, US2002/0009427, and US 2003/0185796 (Wolin *et al.*); WO 2003/061694 (Sing and Siegall); US 2003/0219818 (Bohen *et al.*); US 2003/0219433 and WO 2003/068821 (Hansen *et al.*); US 2003/0219818 (Bohen *et al.*); US2002/0136719 (Shenoy *et al.*); WO 2004/032828 (Wahl *et al.*); and WO 2002/56910 (Hayden-Ledbetter). See also US Patent No. 5,849,898 and EP 330,191 (Seed *et al.*); EP332,865A2 (Meyer and Weiss); US Patent No. 4,861,579 (Meyer *et al.*); US2001/0056066 (Bugelski *et al.*); WO 1995/03770 (Bhat *et al.*); US 2003/0219433 A1 (Hansen *et al.)*; WO 2004/035607 (Teeling *et al.*); US 2004/0093621 (Shitara *et al.*); WO 2004/103404 (Watkins *et al.*); WO 2005/000901 (Tedder *et al.*); US 2005/0025764 (Watkins *et al.*); WO2005/016969 and US 2005/0069545 A1 (Carr *et al.*); WO 2005/014618 (Chang *et al.*). Certain of these include, ***inter alia***, treatment of multiple sclerosis.

Publications concerning therapy with Rituximab include: Perotta and Abuel "Response of chronic relapsing ITP of 10 years duration to Rituximab" Abstract # 3360 *Blood* 10(1)(part 1-2): p.

88B (1998); Stashi *et al.* "Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idopathic thrombocytopenic purpura" *Blood* 98(4):952-957 (2001); Matthews, R. "Medical Heretics" *New Scientist* (7 April, 2001); Leandro *et al.* "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" *Ann Rheum Dis* 61:833-888 (2002); Leandro *et al.* "Lymphocyte depletion in rheumatoid arthritis: early evidence for safety, efficacy and dose response. *Arthritis and Rheumatism* 44(9): S370 (2001); Leandro *et al.* "An open study of B lymphocyte depletion in systemic lupus erythematosus", *Arthritis & Rheumatism* 46(1):2673-2677 (2002); Edwards and Cambridge "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rhematology* 40:205-211 (2001); Edwards *et al.* "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem. Soc. Trans.* 30(4):824-828 (2002); Edwards *et al.* "Efficacy and safety of Rituximab, a B-cell targeted chimeric monoclonal antibody: A randomized, placebo controlled trial in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 46(9): S197 (2002); Levine and Pestronk "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab" *Neurology* 52: 1701-1704 (1999); DeVita *et al.* "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hidashida *et al.* "Treatment of DMARD-Refractory rheumatoid arthritis with Rituximab." Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Tuscano, J. "Successful treatment of Infliximab-refractory rheumatoid arthritis with Rituximab" Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001); Anolik *et al.*,"B lympocyte Depletion in the Treatment of Systemic Lupus (SLE): Phase I/II Trial of Rituximab (RITUXAN®) in SLE" *Arthritis And Rheumatism*, 46(9), S289-S289 Abstract 717 (October, 2002), and Albert *et al.*, "A Phase I Trial of Rituximab (Anti-CD20) for Treatment of Systemic Lupus Erythematosus" *Arthritis And Rheumatism*, 48(12): 3659-3659, Abstract LB9 (December, 2003); Martin and Chan "Pathogenic Roles of B cells in Human Autoimmunity: Insights from the Clinic" *Immunity* 20:517-527 (2004).

Summary of the Invention

The present invention involves, at least in part, the selection of a dose for a CD20 antibody that provides a safe and active treatment regimen in subjects with multiple sclerosis, such as PPMS or RRMS.

Accordingly, the invention concerns a method of treating multiple sclerosis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial

exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

In addition, the invention concerns an article of manufacture comprising: (a) container comprising a CD20 antibody; and (b) a package insert with instructions for treating multiple sclerosis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being administered until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

Brief Description of the Drawings

FIG. 1A is a sequence alignment comparing the amino acid sequences of the light chain variable domain (V_L) of each of murine 2H7 (SEQ ID NO:1), humanized 2H7.v16 variant (SEQ ID NO:2), and the human kappa light chain subgroup I (SEQ ID NO:3). The CDRs of V_L of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:5), and CDR3 (SEQ ID NO:6).

FIG. 1B is a sequence alignment comparing the amino acid sequences of the heavy chain variable domain (V_H) of each of murine 2H7 (SEQ ID NO:7), humanized 2H7.v16 variant (SEQ ID NO:8), and the human consensus sequence of the heavy chain subgroup III (SEQ ID NO:9). The CDRs of V_H of 2H7 and hu2H7.v16 are as follows: CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), and CDR3 (SEQ ID NO:12).

In FIG. 1A and FIG. 1B, the CDR1, CDR2 and CDR3 in each chain are enclosed within brackets, flanked by the framework regions, FR1-FR4, as indicated. 2H7 refers to the 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.

FIG. 2 shows the amino acid sequence of the mature 2H7.v16 light chain (SEQ ID NO:13)

FIG. 3 shows the amino acid sequence of the mature 2H7.v16 heavy chain (SEQ ID NO:14).

FIG. 4 shows the amino acid sequence of the mature 2H7.v31 heavy chain (SEQ ID NO:15). The L chain of 2H7.v31 is the same as for 2H7.v16.

FIG. 5 shows an alignment of the mature 2H7.v16 and 2H7.v511 light chains (SEQ ID NOS. 13 and 16, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

FIG. 6 shows an alignment of the mature 2H7.v16 and 2H7.v511 heavy chains (SEQ ID NOS. 14 and 17, respectively), with Kabat variable domain residue numbering and Eu constant domain residue numbering.

Detailed Description of the Preferred Embodiments

I. Definitions

A "B-cell" is a lymphocyte that matures within the bone marrow, and includes a naive B cell, memory B cell, or effector B cell (plasma cells). The B-cell herein may be a normal or non-malignant B cell.

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.

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.

An "antibody antagonist" herein is a antibody that, upon binding to a B cell surface marker on B cells, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antibody antagonist preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC), inhibition of B-cell proliferation and/or induction of B-cell death (*e.g.* via apoptosis).

"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 that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes that 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 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 that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term 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" refer 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.

"Growth inhibitory" antibodies are those that prevent or reduce proliferation of a cell expressing an antigen to which the antibody binds. For example, the antibody may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

Antibodies that "induce apoptosis" are those that induce programmed cell death, *e.g.* of a B cell, as determined by standard apoptosis assays, such as 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 term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding 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 fragments.

For the purposes herein, an "intact antibody" is one comprising heavy and light variable domains as well as an Fc region.

"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).

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 that 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 that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

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

"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 that 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).

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

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 (immunoglobulins) 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; 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, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

"Humanized" forms of non-human (*e.g.*, murine) 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, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, 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).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region 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" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

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

Examples of antibodies that bind the CD20 antigen include: "C2B8," which is now called "Rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from IDEC Pharmaceuticals, Inc. (US Patent No. 5,736,137; 2B8 deposited with ATCC under accession no. HB11388 on June 22, 1993); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "^{131}I-B1" or "iodine I131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, US Patent No. 5,595,721); 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 (US Patent No. 5,677,180); humanized 2H7; huMax-CD20 (Genmab, Denmark, WO2004/035607); AME-133 (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) or IMMU-106 (US 2003/0219433, Immunomedics); CD20-binding antibodies, including epitope-depleted Leu-16, 1H4, or 2B8, optionally conjugated with IL-2, as in US 2005/0069545A1 and WO 2005/16969 (Carr *et al.*); bispecific antibody that binds CD22 and CD20; for example, hLL2xhA20 (WO2005/14618, Chang *et al.*); 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)); 1H4 (Haisma *et al. Blood* 92:184 (1998)); anti-CD20 auristatin E conjugate (Seattle Genetics); anti-CD20-IL2 (EMD/Biovation/City of Hope); anti-CD20 MAb therapy (EpiCyte); and anti-CD20 antibody TRU 015 (Trubion).

The terms "Rituximab" or "RITUXAN®" herein refer to the genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen and designated "C2B8" in US Patent No. 5,736,137, including fragments thereof that retain the ability to bind CD20. Rituximab is commercially available from Genentech.

Purely for the purposes herein and unless indicated otherwise, "humanized 2H7" refers to a humanized antibody that binds human CD20, or an antigen-binding fragment thereof, wherein the antibody is effective to deplete primate B cells *in vivo*, the antibody comprising in the H chain variable region (V_H) thereof at least a CDR H3 sequence of SEQ ID NO:12 (Fig. 1B) from an anti-human CD20 antibody and substantially the human consensus framework (FR) residues of the human heavy- chain subgroup III (V_HIII). In a preferred embodiment, this antibody further comprises the H chain CDR H1 sequence of SEQ ID NO:10 and CDR H2 sequence of SEQ ID NO:11, and more preferably further comprises the L chain CDR L1 sequence of SEQ ID NO:4, CDR L2 sequence of SEQ ID NO:5, CDR L3 sequence of SEQ ID NO:6 and substantially the human consensus framework (FR) residues of the human light chain subgroup I (VI), wherein the V_H region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3. In a preferred embodiment, such antibody comprises the V_H sequence of SEQ ID NO:8 (v16, as shown in Fig. 1B), optionally also comprising the V_L sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), which may have the amino acid substitutions of D56A and N100A in the H chain and S92A in the L chain (v96). Preferably the antibody is an intact antibody comprising the light and heavy chain amino acid sequences of SEQ ID NOS:13 and 14, respectively, as shown in Figs. 2 and 3. Another preferred embodiment is where the antibody is 2H7.v31 comprising the light and heavy chain amino acid sequences of SEQ ID NOS:13 and 15, respectively, as shown in Figs. 2 and 4. The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC and/or CDC activity, such as one wherein the amino acid substitutions are S298A/E333A/K334A, more preferably 2H7.v31 having the heavy chain amino acid sequence of SEQ ID NO:15 (as shown in Fig. 4). Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that decreases CDC activity, for example, comprising at least the substitution K322A. See U.S. Patent No. 6,528,624B1 (Idusogie *et al.)*.

A preferred humanized 2H7 is an intact antibody or antibody fragment comprising the variable light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG

SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and the variable heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY

NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SS (SEQ ID NO:8).

Where the humanized 2H7 antibody is an intact antibody, preferably it comprises the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

and the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

In the preferred embodiment of the invention, the variable region of variants based on 2H7 version 16 will have 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.

2H7 Version	Heavy chain (V_H) changes	Light chain (V_L) changes	Fc changes
31	-	-	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N10	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L

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An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous 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 "subject" herein is a human subject. Generally, the subject is eligible for treatment for multiple sclerosis. For the purposes herein, such eligible subject is one who is experiencing, has experienced, or is likely to experience, one or more signs, symptoms or other indicators of multiple sclerosis; has been diagnosed with multiple sclerosis, whether, for example, newly diagnosed (with "new onset" MS), previously diagnosed with a new relapse or exacerbation, previously diagnosed and in remission, etc; and/or is at risk for developing multiple sclerosis. One suffering from or at risk for suffering from multiple sclerosis may optionally be identified as one who has been screened for elevated levels of CD20-positive B cells in serum, cerebrospinal fluid (CSF) and/or MS lesion(s) and/or is screened for using an assay to detect autoantibodies, assessed qualitatively, and preferably quantitatively. Exemplary such autoantibodies associated with multiple sclerosis include anti-myelin basic protein (MBP), anti-myelin oligodendrocytic glycoprotein (MOG), anti-ganglioside and/or anti-neurofilament antibodies. Such autoantibodies may be detected in the subject's serum, cerebrospinal fluid (CSF) and/or MS lesion. By "elevated" autoantibody or B cell level(s) herein is meant level(s) of such autoantibodies or B cells which significantly exceed the level(s) in an individual without MS.

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

A "symptom" of MS is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the subject and indicative of MS.

"Multiple sclerosis" refers to the chronic and often disabling disease of the central nervous system characterized by the progressive destruction of the myelin. There are four internationally

recognized forms of MS, namely, primary progressive multiple sclerosis (PPMS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS).

"Primary progressive multiple sclerosis" or "PPMS" is characterized by a gradual progression of the disease from its onset with no superimposed relapses and remissions at all. There may be periods of a leveling off of disease activity and there may be good and bad days or weeks. PPMS differs from RRMS and SPMS in that onset is typically in the late thirties or early forties, men are as likely women to develop it, and initial disease activity is often in the spinal cord and not in the brain. PPMS often migrates into the brain, but is less likely to damage brain areas than RRMS or SPMS; for example, people with PPMS are less likely to develop cognitive problems. PPMS is the sub-type of MS that is least likely to show inflammatory (gadolinium enhancing) lesions on MRI scans. The Primary Progressive form of the disease affects between 10 and 15% of all people with multiple sclerosis. PPMS may be defined according to the criteria in McDonald *et al. Ann Neurol* 50:121–7 (2001). The subject with PPMS treated herein is usually one with probable or definitive diagnosis of PPMS.

"Relapsing-remitting multiple sclerosis" or "RRMS" is characterized by relapses (also known as exacerbations) during which time new symptoms can appear and old ones resurface or worsen. The relapses are followed by periods of remission, during which time the person fully or partially recovers from the deficits acquired during the relapse. Relapses can last for days, weeks or months and recovery can be slow and gradual or almost instantaneous. The vast majority of people presenting with MS are first diagnosed with RRMS. This is typically when they are in their twenties or thirties, though diagnoses much earlier or later are known. Twice as many women as men present with this sub-type of MS. During relapses, myelin, a protective insulating sheath around the nerve fibers (neurons) in the white matter regions of the central nervous system (CNS), may be damaged in an inflammatory response by the body's own immune system. This causes a wide variety of neurological symptoms that vary considerably depending on which areas of the CNS are damaged. Immediately after a relapse, the inflammatory response dies down and a special type of glial cell in the CNS (called an oligodendrocyte) sponsors remyelination - a process whereby the myelin sheath around the axon may be repaired. It is this remyelination that may be responsible for the remission. Approximately 50% of patients with RRMS convert to SPMS within 10 years of disease onset. After 30 years, this figure rises to 90%. At any one time, the relapsing-remitting form of the disease accounts around 55% of all people with MS.

"Secondary progressive multiple sclerosis" or "SPMS" is characterized by a steady progression of clinical neurological damage with or without superimposed relapses and minor remissions and plateaux. People who develop SPMS will have previously experienced a period of RRMS which may have lasted anything from two to forty years or more. Any superimposed relapses and remissions there are, tend to tail off over time. From the onset of the secondary progressive phase

of the disease, disability starts advancing much quicker than it did during RRMS though the progress can still be quite slow in some individuals. After 10 years, 50% of people with RRMS will have developed SPMS. By 25 to 30 years, that figure will have risen to 90%. SPMS tends to be associated with lower levels of inflammatory lesion formation than in RRMS but the total burden of disease continues to progress. At any one time, SPMS accounts around 30% of all people with multiple sclerosis.

"Progressive relapsing multiple sclerosis" refers to "PRMS" is characterized by a steady progression of clinical neurological damage with superimposed relapses and remissions. There is significant recovery immediately following a relapse but between relapses there is a gradual worsening of symptoms. PRMS affects around 5% of all people with multiple sclerosis. Some neurologists believe PRMS is a variant of PPMS.

The expression "effective amount" refers to an amount of the antibody (or other drug) that is effective for preventing, ameliorating or treating the multiple sclerosis. Such an effective amount will generally result in an improvement in the signs, symptoms or other indicators of MS, such as reducing relapse rate, preventing disability, reducing number and/or volume of brain MRI lesions, improving timed 25-foot walk, extending the time to disease progression (*e.g.* using Expanded Disability Status Scale, EDSS), etc.

"Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses administered over a period of time of about 1-20 days. The doses may be given at one time or at fixed or irregular time intervals over this period of exposure. Initial and later (*e.g.* second or third) antibody exposures are separated in time from each other as described in detail herein.

The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, down-regulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (see U.S. Pat. No. 4,665,077); nonsteroidal anti-inflammatory drugs (NSAIDs); ganciclovir, tacrolimus, glucocorticoids such as cortisol or aldosterone, anti-inflammatory agents such as a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist; purine antagonists such as azathioprine or mycophenolate mofetil (MMF); alkylating agents such as cyclophosphamide; bromocryptine; danazol; dapsone; glutaraldehyde (which masks the MHC antigens, as described in U.S. Pat. No. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); hydroxycloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antagonists including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor-alpha antibodies (infliximab or adalimumab), anti-TNF-alpha immunoahesin (etanercept), anti-tumor necrosis factor-beta antibodies, anti-interleukin-2

antibodies and anti-IL-2 receptor antibodies; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 90/08187 published 7/26/90); streptokinase; TGF-beta; streptodornase; RNA or DNA from the host; FK506; RS-61443; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, U.S. Pat. No. 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294; Ianeway, *Nature*, 341: 482 (1989); and WO 91/01133); and T cell receptor antibodies (EP 340,109) such as T10B9.

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, or fragments 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 CYTOXAN cyclosphosphamide; 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); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omega1I (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; 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, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as

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methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestanie, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2;

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LURTOTECAN topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; 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-11, IL-12, IL-15; 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, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "hormone" refers to polypeptide hormones, which are generally secreted by glandular organs with ducts. Included among the hormones are, for example, 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); prolactin, placental lactogen, mouse gonadotropin-associated peptide, inhibin; activin; mullerian-inhibiting substance; and thrombopoietin. As used herein, the term hormone includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence hormone, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "growth factor" refers to proteins that promote growth, and include, for example, hepatic growth factor; fibroblast growth factor; vascular endothelial growth factor; nerve growth factors such as NGF-β; platelet-derived 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; and colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF). As used herein, the term growth factor includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence growth factor, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells and apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits, called alpha and beta, that are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include Alpha6beta1,

Alpha3beta1, Alpha7beta1, LFA-1, alpha 4 integrin etc. As used herein, the term integrin includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence integrin, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody such as Efalizumab (RAPTIVA®) commercially available from Genentech; an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen; diazacyclic phenylalanine derivatives (WO 2003/89410); phenylalanine derivatives (WO 2003/70709, WO 2002/28830, WO 2002/16329 and WO 2003/53926); phenylpropionic acid derivatives (WO 2003/10135); enamine derivatives (WO 2001/79173); propanoic acid derivatives (WO 2000/37444); alkanoic acid derivatives (WO 2000/32575); substituted phenyl derivatives (US Pat. Nos. 6,677,339 and 6,348,463); aromatic amine derivatives (US Pat. No. 6,369,229); and ADAM disintegrin domain polypeptide (US2002/0042368), antibodies to alphavbeta3 integrin (EP 633945); aza-bridged bicyclic amino acid derivatives (WO 2002/02556) etc.

For the purposes herein, "tumor necrosis factor alpha (TNF-alpha)" refers to a human TNF-alpha molecule comprising the amino acid sequence as described in Pennica *et al., Nature,* 312:721 (1984) or Aggarwal *et al., JBC,* 260:2345 (1985).

A "TNF-alpha inhibitor" herein is an agent that inhibits, to some extent, a biological function of TNF-alpha, generally through binding to TNF-alpha and neutralizing its activity. Examples of TNF inhibitors specifically contemplated herein are Etanercept (ENBREL®), Infliximab (REMICADE®) and Adalimumab (HUMIRA™).

Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methrotrexate), azathioprine, D-penicillamine, Gold (oral), Gold (intramuscular), minocycline, cyclosporine, Staphylococcal protein A immunoadsorption, including salts and derivatives thereof, etc.

Examples of "nonsteroidal anti-inflammatory drugs" or "NSAIDs" are acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, including salts and derivatives thereof, etc.

"Corticosteroid" refers to any one of several synthetic or naturally occurring substances with the general chemical structure of steroids that mimic or augment the effects of the naturally occurring corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone), dexamethasone, glucocorticoid and betamethasone.

A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage,

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administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products, etc.

II. Therapy

The present invention provides a method of treating multiple sclerosis in a subject suffering therefrom, comprising administering an effective amount of an antibody that binds to a B-cell surface marker (preferably a CD20 antibody) to the subject. The MS to be treated herein includes primary progressive multiple sclerosis (PPMS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and progressive relapsing multiple sclerosis (PRMS), but therapy of either PPMS or RRMS are the preferred embodiments herein.

According to a preferred dosing protocol, the method comprises administering an effective amount of a CD20 antibody to the MS subject to provide an initial antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams) followed by a second antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams), the second antibody exposure not being provided until from about 16 to 60 weeks from the initial antibody exposure. For purposes of this invention, the second antibody exposure is the next time the subject is treated with the CD20 antibody after the initial antibody exposure, there being no intervening CD20 antibody treatment or exposure between the initial and second exposures.

The interval between the initial and second or subsequent antibody exposures can be measured from either the first or second dose of the initial antibody exposure, but preferably from the first dose of the initial antibody exposure.

In the preferred embodiments herein, the antibody exposures are approximately 24 weeks or 6 months apart; or approximately 48 weeks or 12 months apart.

In one embodiment, the second antibody exposure is not provided until about 20 to 30 weeks from the initial exposure, optionally followed by a third antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 2.5 grams), the third exposure not being administered until from about 46 to 60 weeks (preferably from about 46 to 54 weeks) from the initial exposure, and then, preferably no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.

In an alternative embodiment, the second antibody exposure is not provided until about 46 to 60 weeks from the initial exposure, and subsequent antibody exposures, if any, are not provided until about 46 to 60 weeks from the previous antibody exposure.

Any one or more of the antibody exposures herein may be provided to the subject as a single dose of antibody, or as two separate doses of the antibody (*i.e.*, constituting a first and second dose). The particular number of doses (whether one or two) employed for each antibody exposure is dependent, for example, on the type of MS treated, the type of antibody employed, whether and what type of second medicament is employed, and the method and frequency of administration. Where two separate doses are administered, the second dose is preferably administered from about 3 to 17 days, more preferably from about 6 to 16 days, and most preferably from about 13 to 16 days from the

time the first dose was administered. Where two separate doses are administered, the first and second dose of the antibody is preferably about 0.5 to 1.5 grams, more preferably about 0.75 to 1.3 grams.

In one embodiment, the subject is provided at least about three, or at least four exposures of the antibody, for example, from about 3 to 60 exposures, and more particularly about 3 to 40 exposures, most particularly, about 3 to 20 exposures. Preferably, such exposures are administered at intervals each of approximately 24 weeks or 6 months, or 48 weeks or 12 months. In one embodiment, each antibody exposure is provided as a single dose of the antibody. In an alternative embodiment, each antibody exposure is provided as two separate doses of the antibody. However, not every antibody exposure need be provided as a single dose or as two separate doses.

The antibody may be a naked antibody or may be conjugated with another molecule such as a cytotoxic agent such as a radioactive compound. The preferred antibody herein is Rituximab, humanized 2H7 (*e.g.* comprising the variable domain sequences in SEQ ID NOS. 2 and 8) or humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 23 and 24, or huMax-CD20 (Genmab).

In one embodiment, the subject has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the multiple sclerosis and/or has never been previously treated with an antibody to a B-cell surface marker (*e.g.* never previously treated with a CD20 antibody).

The antibody is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, *e.g.*, US Patent Appln No. 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antibody. Preferably, the dosing is given intravenously, subcutaneously or intrathecally, most preferably by intravenous infusion(s).

While the CD20 antibody may be the only drug administered to the subject to treat the multiple sclerosis, one may optionally administer a second medicament, such as a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody (*e.g.* an LFA-1 antibody such as efalizumab (RAPTIVA®) commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen) etc, with the antibody that binds a B cell surface marker (*e.g.* with the CD20 antibody).

In the preferred embodiment of combination therapy, the antibody is combined with an interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, azathioprine;

intravenous immunoglobulin (gamma globulin); lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, cladribine, total body irradiation, bone marrow transplantation); corticosteroid (*e.g. methylprednisolone, prednisone, dexamethasone,* or glucorticoid), including systemic corticosteroid therapy; non–lymphocyte-depleting immunosuppressive therapy (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class, which includes cerivastatin (BAYCOL®), fluvastatin (LESCOL®), atorvastatin (LIPITOR®), lovastatin (MEVACOR®), pravastatin (PRAVACHOL®), Simvastatin (ZOCOR®); estradiol; testosterone (optionally at elevated dosages; Stuve *et al. Neurology* 8:290–301 (2002)); hormone replacement therapy; treatment for symptoms secondary or related to MS *(e.g.,* spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); plasmapheresis; levothyroxine; cyclosporin A; somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; rehabilitative surgery; radioiodine; thyroidectomy; another B-cell surface antagonist/antibody; etc.

The second medicament is administered with the initial exposure and/or later exposures of the CD20 antibody, such combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

Aside from administration of antibodies to the subject the present application contemplates administration of antibodies by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression administering an "effective amount" of an antibody. See, for example, WO96/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the subject's cells; *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the subject, usually at the site where the antibody is required. For *ex vivo* treatment, the subject's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the subject either directly or, for example, encapsulated within porous membranes that are implanted into the subject (see, *e.g.* U.S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, *etc.* A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

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The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins that bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992). See also WO 93/25673 and the references cited therein.

III. Production of Antibodies

The methods and articles of manufacture of the present invention use, or incorporate, an antibody that binds to a B-cell surface marker, especially one that binds to CD20. Accordingly, methods for generating such antibodies will be described here.

The B cell surface marker to be used for production of, or screening for, antibodies may be, *e.g.*, a soluble form of the marker or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing the marker at their cell surface can be used to generate, or screen for, antibodies. Other forms of the B cell surface marker useful for generating antibodies will be apparent to those skilled in the art.

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention.

(i) Polyclonal antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3

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volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(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 arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal 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); 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 immunoglobulin 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 immunoglobulin 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, 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- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; 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 that 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 that 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 chain variable regions. 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

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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 that 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.

(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 US 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, US Patent Nos. 5,565,332 and 5,573,905.

Human antibodies may also be generated by *in vitro* activated B cells (see US Patents 5,567,610 and 5,229,275).

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. 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; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US 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 B cell surface marker. Other such antibodies may bind the B cell surface marker and further bind a second different B-cell surface marker. Alternatively, an anti-B cell surface marker binding arm may be combined with an arm that 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 B cell. Bispecific antibodies may also be used to localize cytotoxic agents to the B cell. These antibodies possess a B cell surface marker-binding arm and an arm that 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).

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 US Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers that 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 (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089).

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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 US Patent No. 4,676,980, along with a number of cross-linking techniques.

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

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 that 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).

IV. Conjugates and Other Modifications of the Antibody

The antibody used in the methods or included in the articles of manufacture herein is optionally conjugated to a cytotoxic agent. For instance, the antibody may be conjugated to a drug as described in WO2004/032828.

Chemotherapeutic agents useful in the generation of such antibody-cytotoxic agent conjugates have been described above.

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Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (US Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one 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 conjugate.

Alternatively, the antibody is 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 that 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)).

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

The present invention further contemplates antibody conjugated with 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 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 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 subject, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) that is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

The antibodies of the present invention may also be conjugated with a prodrug-activating enzyme that converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of such conjugates includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the antibody by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

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 (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody fragments, such as Fab', linked to one or more PEG molecules are an especially preferred embodiment of the invention.

The antibodies disclosed herein may also be formulated as liposomes. 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 an 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).

Amino acid sequence modification(s) of the antibody 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

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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 an 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 of an enzyme, or a polypeptide that 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 different residue. The sites of greatest interest for substitutional mutagenesis of antibody antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a 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. 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 is 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 in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to 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. Such altering includes 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 polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The 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) concerning a CD20 antibody composition. Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc

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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.

The preferred glycosylation variant herein comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US Pat. Appl. No. US 2003/0157108 A1, Presta, L; WO 00/61739A1; WO01/29246A1; US2003/0115614A1; US2002/0164328A1; US2004/0093621A1; US2004/0132140A1; US2004/0110704A1; US2004/0110282A1; US2004/0109865A1; WO03/085119A1; WO03/084570A1; WO2005/035778; WO2005/035586 (describing RNA inhibition (RNAi) of fucosylation); Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004)).

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

It may be desirable to 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 an antibody 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 that 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.*).

V. Pharmaceutical Formulations

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

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sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Exemplary anti-CD20 antibody formulations are described in WO98/56418. This publication describes a liquid multidose formulation comprising 40 mg/mL Rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8BC. Another anti-CD20 formulation of interest comprises 10mg/mL Rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5.

Lyophilized formulations adapted for subcutaneous administration are described in US Pat No. 6,267,958 (Andya *et al.*). Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

Crystalized forms of the antibody or antibody are also contemplated. See, for example, US 2002/0136719A1 (Shenoy *et al.*).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent; chemotherapeutic agent; immunosuppressive agent; cytokine; cytokine antagonist or antibody; growth factor; hormone; integrin; integrin antagonist or antibody (*e.g.* an LFA-1 antibody such as efalizumab/RAPTIVA commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab/TYSABRI®) available from Biogen); interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; testosterone; hormone replacement therapy; drug that treats symptoms secondary or related to MS (*e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid (*e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A; somatastatin analogue; cytokine antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody (*e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4 integrin antibody such as natalizumab); or another B-cell surface antagonist/antibody; etc in the formulation. The type and effective amounts of such other agents depend, for example, on the amount of antibody present in the formulation, the type of multiple sclerosis being treated, and clinical parameters of the subjects. These are generally used in

the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

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

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

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

VI. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of multiple sclerosis described above is provided. Preferably, the article of manufacture comprises:(a) a container comprising a composition comprising an antibody that binds to a B-cell surface marker (*e.g.* a CD20 antibody) and a pharmaceutically acceptable carrier or diluent within the container; and (b) a package insert with instructions for administering the composition to a subject suffering from multiple sclerosis to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial exposure; or instructions for administering the composition to a subject suffering from PPMS.

The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for treating the multiple sclerosis and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the antibody. The label or package insert indicates that the composition is used for treating multiple sclerosis in a subject suffering therefrom with specific guidance regarding dosing amounts and

intervals of antibody and any other drug being provided. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Optionally, the article of manufacture herein further comprises a container comprising an agent other than the antibody for treatment and further comprising instructions on treating the mammal with such agent, such agent preferably being a chemotherapeutic agent or immunosuppressive agent, interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting drug (*e.g.*, mitoxantrone, cyclophosphamide, Campath, anti-CD4, or cladribine); non–lymphocyte-depleting immunosuppressive drug (*e.g.*, mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class; estradiol; hormone replacement therapy; drug that treats symptoms secondary or related to MS (*e.g.*, spasticity, incontinence, pain, fatigue); a TNF inhibitor; disease-modifying anti-rheumatic drug (DMARD); non-steroidal anti-inflammatory drug (NSAID); corticosteroid (*e.g.* methylprednisolone, prednisone, dexamethasone, or glucorticoid); levothyroxine; cyclosporin A; somatastatin analogue; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; integrin antagonist or antibody (*e.g.* an LFA-1 antibody, such as efalizumab or an alpha 4 integrin antibody such as natalizumab); and another B-cell surface marker antibody; etc.

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1

TREATMENT OF PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS (PPMS)

A subject with diagnosis of PPMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) is treated with a CD20 antibody in this example.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 24 (Day 169), Week 48 (Day 337), and Week 72 (Day 505). The second infusion of the subsequent courses of treatment will be 14 ∀ 1 days after the first infusion.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse is defined as all of the following:

- An acute appearance of a neurologic abnormality that persists for at least 24 hours
- A change not attributable to fever, infection, trauma, concomitant medications, or other etiology
- An event with objective change on examination by the blinded examining investigator, including a minimum of 1-point change on one of the following FS scales: pyramidal, cranial nerves, cerebellar, sensory, vision, or gait

The following regimen of corticosteroids may be used: 1 g IV methylprednisolone daily for 3 days, followed by 60 mg prednisone daily for 5 days, and decreasing by 10-mg increments each day thereafter. If IV methylprednisolone is not available, then 150 mg IV dexamethasone daily for 3 days may be substituted. Only one course of corticosteroids should be administered for an exacerbation. Subsequent immunological studies and MRI scans should be obtained at least 4 weeks after completion of the corticosteroid regimen. Corticosteroid inhalers (oral and nasal) or intra-articular injections may be used.

Additional treatments to be optionally combined with the CD20 antibody include IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

The primary efficacy outcome measure is the time to confirmed disease progression. Disease progression is defined as an increase of ∃ 1.0 point from baseline Expanded Disability Status Scale (EDSS) (Kurtzke J. *Neurology* 33(11):1444–52 (1983)), if the baseline EDSS is between 2.0 and 5.5 points (inclusive), or an increase of ∃ 0.5 point if the baseline EDSS is ∃ 5.5 points (inclusive), for which change is not attributable to another etiology (*e.g.*, fever, concurrent illness, MS relapse or exacerbation, or concomitant medication). Confirmation of disease progression may occur at a regularly scheduled visit that is at least 12 weeks (84 days) after the initial progression.

Secondary efficacy outcome measures include:

- Change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan
- Change from baseline to Week 96 in brain volume on brain MRI scan

Optionally, improvement in any one or more of:

- Multiple Sclerosis Functional Composite Scale (MSFCS)
- EDSS
- Proportion of subjects with confirmed disease progression at Week 96, as determined using EDSS
- Upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS)
- Ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS)
- Cognition, as measured by the Paced Auditory Serial Addition Test (3 seconds only; a subscale of the MSFCS)
- Total volume of brain T2 lesions on MRI scans (Weeks 48 and 122)
- Total volume of brain T1 lesions on MRI scans
- Cross sectional area of the cervical spinal cord on MRI scans
- Brain volume on MRI scans (Weeks 48 and 122)

The subject treated with Rituximab as described herein will show improvement in the signs, symptoms or other indicators of PPMS according to any one or more of the above outcome measures.

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EXAMPLE 2

TREATMENT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS

Subjects with RRMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) are treated with a CD20 antibody in this example, where the antibody exposures are approximately 6 months apart.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 24 (Day 169), Week 48 (Day 337), and Week 72 (Day 505). The second infusion of the subsequent courses of treatment will be 14 ∀ 1 days after the first infusion.

Preferably Rituximab is the only agent administered to treat the RRMS. However, subjects may optionally receive IV or oral corticosteroids, IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse in this Example is defined as:

The appearance of new or recurrent neurological symptoms consistent with MS lasting more than 48 hours in a subject who has been in a relatively stable or improving neurologic state for at least 30 days. The change in neurologic symptoms must be accompanied by objective neurologic worsening consistent with an increase of at least half a step on the EDSS, or 2 points on one of the appropriate functional system scores (FSS), or 1 point on two or more of the appropriate FSS. The change must be verified by the examining investigator and must affect the selected FS scales (*i.e.*, pyramidal, gait, cerebellar, brainstem, sensory, or visual). Symptoms must persist for≥ 24 hours and should not be attributable to confounding clinical factors (*e.g.*, fever, infection, injury, adverse reactions to concomitant medications). A single episode of a paroxysmal symptom (*e.g.*, tonic spasm) is not a relapse, but the new onset of multiple occurrences of a paroxysmal symptom over at least 24 hours can be a relapse if accompanied by new, corresponding objective manifestations. Sensory symptoms with no change on clinical examination, fatigue, mood change, or bladder or bowel urgency or incontinence will not be sufficient to establish a relapse.

The primary efficacy outcome measure is the MRI endpoint of gadolinium-enhancing lesions, or time to confirmed disease progression (defined as an increase of ∃ 1.0 point from baseline Expanded Disability Status Scale (EDSS); Kurtzke J. *Neurology* 33(11):1444–52 (1983)). The primary efficacy endpoint may be the total number of gadolinium-enhancing T1 lesions observed on serial MRI scans of the brain at Weeks 12, 16, 20, and 24.

Secondary efficacy outcome measures include frequency of relapse; change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan (*e.g.* change in total volume of T2 lesions on MRA scans of the brain from screening to weeks 24 and 36); change from baseline to Week 96 in brain volume on brain MRI scan; Multiple Sclerosis Functional Composite Scale (MSFCS) and its subscales; upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS); ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS); cognition, as measured by the Paced Auditory Serial Addition Test (a subscale of the MSFCS); Multiple Sclerosis Quality of Life–54 (MSQOL-54) questionnaire; total volume of brain T1 lesions on MRI scans (*e.g.* total number of gadolinium-enhancing T1 lesions observed on serial MRA scans of the brains at weeks 20, 28, and 36); cross sectional area of the cervical spinal cord on MRI scans; proportion of subjects relapsing by weeks 24 (*i.e* between week 0 and week 24) and 36 (*i.e.* between week 0 and week 36); the Combined Unique Activity Measure at week 24 and 36.

The patient treated with Rituximab as described above will display an improvement in any one or more of the above outcome measures.

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EXAMPLE 3

TREATMENT OF RELAPSING-REMITTING MULTIPLE SCLEROSIS

A subject with RRMS as defined by McDonald *et al. Ann Neurol* 50:121–7 (2001) is treated with a CD20 antibody herein. In this example, the antibody exposures are approximately 1 year apart.

Rituximab, commercially available from Genentech, is formulated for IV administration as a sterile product in 9.0 mg/mL sodium chloride, 0.7 mg/mL polysorbate 80, 7.35 mg/mL sodium citrate dehydrate, and Sterile Water for Injection (pH 6.5).

The first course of treatment will consist of a dose of 1 g intravenous (IV) Rituximab administered on each of Days 1 and 15. Subjects will receive acetaminophen (1 g) and diphenhydramine HCl (50 mg), or equivalent, by mouth 30–60 minutes prior to the start of each infusion.

Subsequent courses of treatment will be administered starting at Week 48, and Week 96. The second infusion of the subsequent courses of treatment will be 14 ∀ 1 days after the first infusion.

Preferably Rituximab is the only agent administered to treat the RRMS. However, subjects may optionally receive IV or oral corticosteroids, IFN-beta, glatiramer acetate, methotrexate, cyclophosphamide, or mitoxantrone.

Subjects who experience a first relapse may receive rescue treatment with IV or oral corticosteroids. Systemic corticosteroids may be administered using a regimen that does not exceed exposure or duration of treatment appropriate for an MS relapse. A relapse in this Example is defined as:

The appearance of new or recurrent neurological symptoms consistent with MS lasting more than 48 hours in a subject who has been in a relatively stable or improving neurologic state for at least 30 days. The change in neurologic symptoms must be accompanied by objective neurologic worsening consistent with an increase of at least half a step on the EDSS, or 2 points on one of the appropriate functional system scores (FSS), or 1 point on two or more of the appropriate FSS. The change must be verified by the examining investigator and must affect the selected FS scales (*i.e.*, pyramidal, gait, cerebellar, brainstem, sensory, or visual). Symptoms must persist for≥ 24 hours and should not be attributable to confounding clinical factors (*e.g.*, fever, infection, injury, adverse reactions to concomitant medications). A single episode of a paroxysmal symptom (*e.g.*, tonic spasm) is not a relapse, but the new onset of multiple occurrences of a paroxysmal symptom over at least 24 hours can be a relapse if accompanied by new, corresponding objective manifestations. Sensory symptoms with no change on clinical examination, fatigue, mood change, or bladder or bowel urgency or incontinence will not be sufficient to establish a relapse.

The primary efficacy outcome measure is the MRI endpoint of gadolinium-enhancing lesions, or time to confirmed disease progression (defined as an increase of ∃ 1.0 point from baseline Expanded Disability Status Scale (EDSS); Kurtzke J. *Neurology* 33(11):1444–52 (1983)). The primary efficacy endpoint may be the total number of gadolinium-enhancing T1 lesions observed on serial MRI scans of the brain at Weeks 12, 16, 20, and 24.

Secondary efficacy outcome measures include frequency of relapse; change from baseline to Week 96 in the total volume of T2 lesions on brain MRI scan (*e.g.* change in total volume of T2 lesions on MRA scans of the brain from screening to weeks 24 and 36); change from baseline to Week 96 in brain volume on brain MRI scan; Multiple Sclerosis Functional Composite Scale (MSFCS) and its subscales; upper extremity function, as measured by the 9-Hole Peg Test (a subscale of the MSFCS); ambulation, as measured by the Timed 25-Foot Walk (a subscale of the MSFCS); cognition, as measured by the Paced Auditory Serial Addition Test (a subscale of the MSFCS); Multiple Sclerosis Quality of Life–54 (MSQOL-54) questionnaire; total volume of brain T1 lesions on MRI scans (*e.g.* total number of gadolinium-enhancing T1 lesions observed on serial MRA scans of the brains at weeks 20, 28, and 36); cross sectional area of the cervical spinal cord on MRI scans; proportion of subjects relapsing by week 24 (*i.e* between week 0 and week 24) and week 36 (*i.e.* between week 0 and week 36); the Combined Unique Activity Measure at week 24 and 36.

The patient treated with the above with Rituximab will display an improvement in any one or more of the above outcome measures.

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EXAMPLE 4

HUMANIZED 2H7 VARIANTS

This example describes humanized 2H7 antibody variants for use in the methods disclosed herein. The humanized 2H7 antibody preferably 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. 18), for example SEQ ID NO:4 (Fig. 1A),

CDR L2 sequence of SEQ ID NO:5 (Fig. 1A),

CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID NO. 19), for example SEQ ID NO:6 (Fig. 1A),

CDR H1 sequence of SEQ ID NO:10 (Fig. 1B),

CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID NO. 20), for example SEQ ID NO:11 (Fig. 1B), and

CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W or D, and the X as position 7 is S or R (SEQ ID NO. 21), for example SEQ ID NO:12 (Fig. 1B).

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:8 (v16, as shown in Fig. 1B), optionally also comprising the variable light domain sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), 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. 13 or 16, and heavy chain amino acid sequences of SEQ ID NO. 14, 15, 17, 22 or 25.

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:2 and the variable heavy domain of SEQ ID NO:8, 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:8 and a variable light domain with alteration M32L; or S92A; or M32L and S92A; in SEQ ID NO:2.

M34 in the variable heavy domain of 2H7.v16 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 2H7.v16 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 2H7.v16 variable light domain sequence:

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DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and 2H7.v16 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SS (SEQ ID NO:8).

Where the humanized 2H7.v16 antibody is an intact antibody, it may comprise the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13);

and the heavy chain amino acid sequence of SEQ ID NO. 14 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPG (SEQ ID NO:22).

Another preferred humanized 2H7 antibody comprises 2H7.v511 variable light domain sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:23) and 2H7.v511 variable heavy domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGN GATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQ GTLVTVSS (SEQ ID NO. 24).

Where the humanized 2H7.v511 antibody is an intact antibody, it may comprise the light chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:16)

and the heavy chain amino acid sequence of SEQ ID NO. 17 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR
VVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO. 25).

WHAT IS CLAIMED IS:

1. A method of treating multiple sclerosis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being provided until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

2. The method of claim 1 wherein the second exposure is not provided until from about 20 to 30 weeks from the initial exposure.

3. The method of claim 1 or 2 wherein the initial and second antibody exposures are each provided in amounts of about 1.5 to 2.5 grams.

4. The method of any one of claims 2-3 additionally comprising administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure of about 0.5 to 4 grams, the third exposure not being administered until from about 46 to 60 weeks from the initial exposure.

5. The method of claim 4 wherein the third antibody exposure is provided in an amount of about 1.5 to 2.5 grams.

6. The method of claim 4 or 5 wherein the third exposure is not provided until from about 46 to 54 weeks from the initial exposure.

7. The method of any one of claims 4-6 wherein no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.

8. The method of claim 1 wherein the second exposure is not provided until from about 46 to 60 weeks from the initial exposure.

9. The method of any one of claims 1-8 wherein each of the antibody exposures is provided to the subject as one dose of antibody.

10. The method of any one of claim 1-8 wherein each of the antibody exposures is provided to the subject as two doses of antibody, wherein the two doses constitute a first dose and a second dose.

11. The method of claim 10 wherein the second dose is administered from about 3-17 days from the time the first dose was administered.

12. The method of claim 11 wherein the second dose is administered from about 6-16 days from the time the first dose was administered.

13. The method of claim 12 wherein the second dose is administered from about 13-16 days from the time the first dose was administered.

14. The method of any one of claims 10-13 wherein the first and second dose of antibody are each about 0.5 to 1.5 grams.

15. The method of any one of claims 10-14 wherein the first and second dose of antibody are each about 0.75 to 1.3 grams.

16. The method of any one of the claims 1-15 wherein three or more antibody exposures are administered to the subject.

17. The method of any one of claims 1-16 wherein four or more antibody exposures are administered to the subject

18. The method of any one of claims 1-17 wherein a second medicament is administered with the initial exposure or later exposures, wherein the CD20 antibody is a first medicament.

19. The method of claim 18 wherein the second medicament is selected from the group consisting of an interferon, glatiramer acetate, a cytotoxic agent, chemotherapeutic agent, mitoxantrone, methotrexate, cyclophosphamide, chlorambucil, azathioprine, gamma globulin, Campath, anti-CD4, cladribine, corticosteroid, mycophenolate mofetil (MMF), cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone replacement drug, a TNF inhibitor, disease-modifying anti-rheumatic drug (DMARD), non-steroidal anti-inflammatory drug (NSAID), levothyroxine, cyclosporin A, somatastatin analogue, cytokine or cytokine receptor antagonist, anti-metabolite, immunosuppressive agent, integrin antagonist or antibody, LFA-1 antibody, efalizumab, alpha 4 integrin antibody, natalizumab, and another B-cell surface marker antibody.

20. The method of any one of claims 1-19 wherein the multiple sclerosis is relapsing-remitting multiple sclerosis (RRMS).

21. The method of any one of claims 1-19 wherein the multiple sclerosis is primary progressive multiple sclerosis (PPMS).

22. The method of any one of claims 1-21 wherein the subject has never been previously treated with a CD20 antibody.

23. The method of any one of claims 1-22 wherein the antibody is a naked antibody.

24. The method of any one of claims 1-22 wherein the antibody is conjugated with another molecule.

25. The method of claim 24 wherein the other molecule is a cytotoxic agent.

26. The method of any one of claims 1-25 wherein the antibody is administered intravenously.

27. The method of claim 26 wherein the antibody is administered intravenously for each antibody exposure.

28. The method of any one of claims 1-25 wherein the antibody is administered subcutaneously.

29. The method of any one of claims 1-25 wherein the antibody is administered intrathecally.

30. The method of any one of claims 1-29 wherein the CD20 antibody is the only medicament administered to the subject to treat the multiple sclerosis.

31. The method of any one of claims 1-30 wherein the antibody is Rituximab.

32. The method of any one of claims 1-30 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 2 and 8.

33. The method of any one of claims 1-30 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS. 23 and 24.

34. The method of any one of claims 1-32 wherein the subject has elevated anti-myelin basic protein (MBP), anti-myelin oligodendrocyte glycoprotein (MOG), anti-ganglioside and/or or anti-neurofilament antibody level(s).

35. The method of any one of claims 1-33 wherein elevated levels of B cells are present in cerebrospinal fluid (CSF), multiple sclerosis lesion, or serum of the subject.

36. An article of manufacture comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating multiple sclerosis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, the second exposure not being administered until from about 16 to 60 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as one or two doses of antibody.

37. The article of claim 36 further comprising a container comprising a second medicament, wherein the CD20 antibody is a first medicament, and further comprising instructions on the package insert for treating the subject with the second medicament.

38. The article of claim 37 wherein the second medicament is selected from the group consisting of an interferon, glatiramer acetate, a cytotoxic agent, chemotherapeutic agent, mitoxantrone, methotrexate, cyclophosphamide, chlorambucil, azathioprine, gamma globulin, Campath, anti-CD4, cladribine, corticosteroid, mycophenolate mofetil (MMF), cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone replacement drug, a TNF inhibitor, disease-modifying anti-rheumatic drug (DMARD), non-steroidal anti-inflammatory drug (NSAID), levothyroxine, cyclosporin A, somatastatin analogue, cytokine or cytokine receptor antagonist, anti-metabolite, immunosuppressive agent, and another B-cell surface marker antibody.

METHOD FOR TREATING MULTIPLE SCLEROSIS

ABSTRACT OF THE DISCLOSURE

Methods for treating multiple sclerosis (MS) with a CD20 antibody using special dosing regimens and protocols are described. Articles of manufacture for use in such methods are also described.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
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Washington, D.C. 20231

SEPTEMBER 03, 2004

PTAS

700108444

GENENTECH, INC.
WENDY M. LEE
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 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 08/20/2004 REEL/FRAME: 015075/0988
NUMBER OF PAGES: 2

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
FROHNA, PAUL A. DOC DATE: 08/16/2004

ASSIGNEE:
GENENTECH INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080-4990

SERIAL NUMBER: 60576993 FILING DATE: 06/04/2004
PATENT NUMBER: ISSUE DATE:
TITLE: METHOD FOR TREATING MULTIPLE SCLEROSIS

314

Docket No. PR2134

ASSIGNMENT

WHEREAS, Paul A. Frohna, a citizen of US , residing at 3860 19th Street, San Francisco, CA 94114 (hereinafter "ASSIGNOR"), has invented a new and useful invention in

METHOD FOR TREATING MULTIPLE SCLEROSIS

for which a provisional application Serial No. 60/576,993, if received (Docket No. PR2134) has been filed by him on June 4, 2004; and

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

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

That for good and valuable consideration the receipt of which is hereby acknowledged, the said ASSIGNOR 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 ASSIGNOR covenants and agrees 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 ASSIGNOR 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 ASSIGNOR 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 ASSIGNOR, their respective heirs, legal representatives and assigns.

Said ASSIGNOR hereby warrants and represents that he has not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF I undersign as follows;

South San Francisco.

Dated: 16 Aug 2004

Paul A. Frohna, MD PhD

Paul A. Frohna

NO: 99708

315

State of California

SECRETARY OF STATE

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. *Country: United States of America*
   This public document
2. *has been signed by AIDA MICLAT GUIAM*
3. *acting in the capacity of Notary Public, State of California*
4. *bears the seal/stamp of AIDA MICLAT GUIAM, Notary Public, State of California*

CERTIFIED

5. *At Sacramento, California*
6. *the 15th day of December 2005*
7. *by Deputy Secretary of State, State of California*
8. *No. 267083*
9. *Seal/Stamp:*

10. *Signature*

En la Notaría Cuarenta de [illegible] entejó la presente fotocopia [illegible] el original que se [illegible]

12 OCT. 2006

NOTARIO CUARENTA [illegible]

Norby Fernando [illegible]

Bogotá, D.C. [illegible]

Secretary of State

BY

NP-84 A (REV. 4-00)

OSP 05 60772

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California
County of San Mateo } ss.

On December 6, 2005 (Date) before me, Aida Miclat Guiam (Name and Title of Officer (e.g., "Jane Doe, Notary Public"))

personally appeared Timothy R. Schwartz (Name(s) of Signer(s))

☐ personally known to me
☒ proved to me on the basis of satisfactory evidence

to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal.

Aida Miclat Guiam
Signature of Notary Public

AIDA MICLAT GUIAM
Commission # 1458662
Notary Public - California
San Mateo County
My Comm. Expires Dec 9, 2007

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Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

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☐ Corporate Officer — Title(s): _____
☐ Partner — ☐ Limited ☐ General
☐ Attorney-In-Fact
☐ Trustee
☐ Guardian or Conservator
☐ Other: _____

Signer Is Representing: _____

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12 OCT. 2006
NOTAZIO CUARENTA DE
Mesby Fernando Mora [illegible]
Bogotá, D.C., Colombia

Brigard & Castro
BOGOTA - COLOMBIA

317

PODER

Yo (nosotros)

domiciliado(s) en

Nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **BEATRIZ JARAMILLO** y/o **CARLOS H. PALACIO EASTMAN** y/o **HECTOR GOMEZ MEJIA**, de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(a) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeren conducentes al resguardo de mis (nuestros intereses), con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer, y la de sustituirlo en todo o en parte y revocar sustituciones. Asimismo nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **BEATRIZ JARAMILLO** y/o **CARLOS H. PALACIO EASTMAN** y/o **HECTOR GOMEZ MEJIA**,, domiciliado(a) en la Carrera 7 No. 16-56 Piso 9, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales.

Otorgado y firmado en

hoy de de 200_

Firma

Nombre

Cargo

Es la fiel copia tomada del original que se ha tenido a la vista

12 OCT. 2006

NOTARIO CUARENTA

Notaría Cuarenta de Bogotá, D.C.

POWER OF ATTORNEY

I (We) **Genentech, Inc.**
1 DNA Way

domiciled in **South San Francisco, California 94080-4990 USA**

Hereby appoints **JUAN PABLO CADENA SARMIENTO** and/or **BEATRIZ JARAMILLO** and/or **CARLOS H. PALACIO EASTMAN** and/or **HECTOR GOMEZ MEJIA**, of Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names and emblems, registration of copyright and contracts related thereto, certificates of obtainers of vegetables varieties, as well as extensions, renewals and recordal of assignments and changes of name and domicile related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or exploitation of licenses, action of unfair competition; criminal proceedings, actions for payment of damages: claims or observations on patent applications, models, industrial designs and utility models; in the exercise of preventive measures and in other actions or similar proceedings.

To that effect they hereby empowered to file applications, formulate descriptions, amendments, protests, declarations, oppositions, cancellations, nullities, appeals and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights granted or in the process of granting; to receive all kinds of documents and valuables, giving the respective release of receipt, and, in general, to take before said authorities the necessary steps to such effect, and any measures that they may deem pertinent to the safeguard of my (our) interest(s) with all the other necessary legal powers.

This power includes the faculty to ratify or confirm desist, settle and compromise on my (our) behalf, and to substitute this power in whole or in part and to revoke substitutions. At the same time I (We) hereby appoints **JUAN PABLO CADENA SARMIENTO** and/or **BEATRIZ JARAMILLO** and/or **CARLOS H. PALACIO EASTMAN** and/or **HECTOR GOMEZ MEJIA**, domiciled in Carrera 7 No. 16-56, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

Granted and signed in **South San Francisco, California USA**

this 6th day of December 2005

Signature

Name Timothy R. Schwartz, Ph.D.
Title Section Patent Counsel
Authorized Corporate Signatory
Genentech, Inc.

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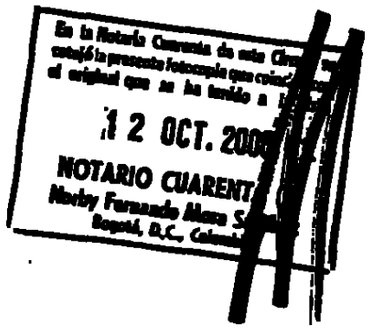
AUTHORIZATION

Genentech, Inc. hereby authorizes any of Janet E. Hasak, Mark T. Kresnak, Jeffrey S. Kubinec and Timothy R. Schwartz, for so long as they remain employees of Genentech, Inc., to execute, on its behalf, any submissions associated with the filing, prosecution, granting, and extension and maintenance of grant of patent and trademark applications in the U.S. and foreign countries, contracts relating to biological deposits with the American Type Culture Collection, and assignments to publishers of copyrights covering works authored by Genentech employees.

August 27, 2002
Dated

Sean Johnston
Sean A. Johnston
Vice President,Intellectual Property
Genentech, Inc.



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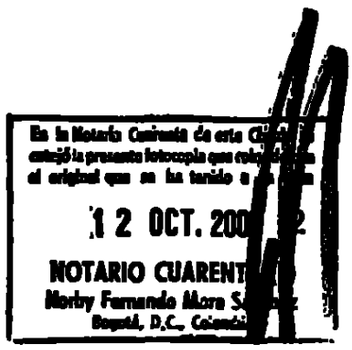
ESTADO DE CALIFORNIA
SECRETARIA DE ESTADO

APOSTILLA
(Convención de La Haya del 5 de Octubre de 1961)

1 País: Estados Unidos de América
Esta documento público
2. ha sido firmado por AIDA MICLAT GUIAM
3. actuando en capacidad de Notario Público, Estado de California
4. lleva el sello/timbre de AIDA MICLAT GUIAM, Notario Público Estado de California

CERTIFICADO

5. en Sacramento, California
6. el 15 de diciembre de 2005
7. por el Secretario de Estado Delegado, Estado de California
8. No. 267083
9. Sello/Timbre



10. Firma

(Firma)
Secretario de Estado

320

RECONOCIMIENTO TODO-PROPOSITO DE CALIFORNIA

Estado de California)
} ss.
Condado de San Mateo)

El 6 de diciembre de 2005, ante mi, AIDA MICLAT GUIAM, apareció personalmente Thimoty R. Schwartz

☐ a quien conozco personalmente

☒ quien demostró ante mi en base a evidencia satisfactoria ser la persona cuyo nombre esta suscrito en el instrumento y quien reconoció ante mi que ejecutó el mismo en su capacidad autorizada, y que por su firma en el instrumento la persona o la entidad a nombre de la cual la persona actuó, ejecutó el instrumento.

Hay un sello que dice:
AIDA MICLAT GUIAM
COMISIÓN # 14556612
Notario Público-California
Condado de San Mateo
Mi comisión expira Dic. 9 de 2007

Atestigua mi firma y sello oficial,

AIDA MICLAT GUIAM

OPCIONAL

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☐ Funcionario Corporativo – Título(s): _____
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☐ Abogado de hecho
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☐ Guardián o conservador
☐ Otro: _____

El firmante representa: _____

Huella índice derecho del firmante
Parte alta del dedo aquí

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12 OCT. 2006

NOTARIO CUARENTA

Norby Fernando Mora Sánchez

Bogotá, D.C., Colombia

250 321

AUT RIZACION

Genentech, Inc. por la presente autoriza a cualquiera de Janet E. Hasak, Mark T. Kresnak, Jeffrey S. Kubinec y Thomity R. Schwartz, mientras permanezcan como empleados de Genentech, Inc., a ejecutar, a su nombre, cualquier solicitud asociada con presentación, procesamiento, consecución, y extensión y mantenimiento de concesión de solicitudes de patente y marca registrada en los Estados Unidos y países extranjeros, contratos relacionados con depósitos biológicos con la Colección Americana de Tipo de Cultivo, y cesiones a publicistas de derechos de autor que cubran los trabajos de autoría de los empleados de Genentech.

27 de Agosto de 2002
Fechado

(Firma)
Sean A. Johnston
Vicepresidente, Propiedad Intelectual
Genentech, Inc.

97.200
114

322

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

En la Notaría Cuarenta de esta C... cotejó la presente fotocopia que coin... el original que se ha tenido a ...

12 OCT. 2006

NOTARIO CUARENTA

Nerby Fernando Mora Sánch...

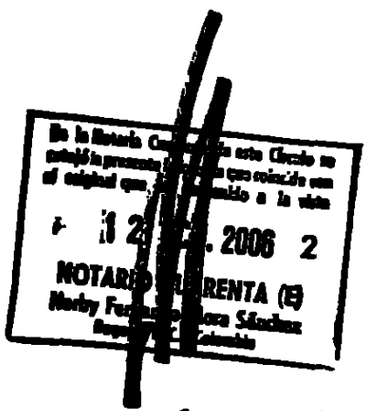
Bogotá, D.C., Colombia

Delaware

323

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE RESTATED CERTIFICATE OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE TWENTY-SECOND DAY OF JULY, A.D. 1999, AT 9 O'CLOCK A.M.



Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900384

DATE: 05-24-05

2004
324

AMENDED AND RESTATED

CERTIFICATE OF INCORPORATION

GENENTECH, INC.

Genentech, Inc. (the "Corporation"), a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware, does hereby amend the Certificate of Incorporation of the Corporation, which was originally filed on December 23, 1986 under the name "Genentech Delaware, Inc.", and subsequently amended.

The Certificate of Incorporation of the Corporation is hereby restated and further amended to read in its entirety as follows:

ARTICLE 1

The name of the Corporation is: Genentech, Inc.

ARTICLE 2

The address of the registered office of the Corporation in the State of Delaware is Corporation Service Company, 1013 Centre Road, City of Wilmington, County of New Castle, Delaware 19805. The name of its registered agent at such address is Corporation Service Company.

ARTICLE 3

The purpose of the Corporation is to engage in any lawful act or activity for which corporations may be organized under the General Corporation Law of the State of Delaware as the same exists or may hereafter be amended ("Delaware Law").

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 09:00 AM 07/22/1999
991302331 – 2112549

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ARTICLE 4

SECTION 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is four hundred million (400,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Three hundred million (300,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock") The Common Stock of the Corporation shall be all of one class.

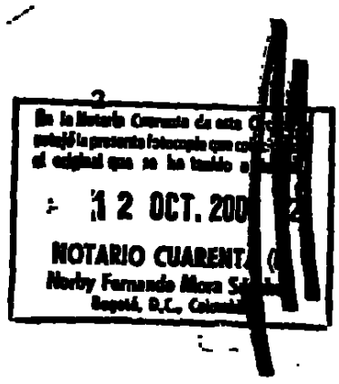
(b) The number of authorized shares of any class or classes of stock may be increased or decreased (but not below the number of shares thereof then outstanding) by the affirmative vote of a majority in voting power of the outstanding stock of the Corporation entitled to vote thereon irrespective of the provisions of Section 242(b)(2) of Delaware Law.

SECTION 4.02. *Common Stock.* (a) *Issuance and Consideration.* Any unissued or treasury shares of the Common Stock may be issued for such consideration as may be fixed in accordance with applicable law from time to time by the Board of Directors.

(b) *Dividends.* Subject to the rights of holders of the Preferred Stock, the holders of the Common Stock shall be entitled to receive, when and as declared by the Board of Directors, out of the assets of the Corporation which are by law available therefor, dividends payable either in cash, in property, or in shares of stock and the holders of the Preferred Stock shall not be entitled to participate in any such dividends (unless otherwise provided by the Board of Directors in any resolution providing for the issue of a series of Preferred Stock).

SECTION 4.03. *Preferred Stock.*

(a) *Series and Limits of Variations between Series.* Any unissued or treasury shares of the Preferred Stock may be issued from time to time in one or more series for such consideration as may be fixed from time to time by the Board of Directors. Before any shares of Preferred Stock of any particular series shall be issued, a certificate shall be filed with the Secretary of State of Delaware setting forth the designation, rights, privileges, restrictions, and conditions to be attached to the Preferred Stock of such series and such other matters as may be required, and the Board of Directors shall fix and determine, and is hereby expressly empowered to fix and determine, in the manner provided by law, the particulars of



the shares of such series (so far as not inconsistent with the provisions of this Article 4 applicable to all series of Preferred Stock), including, but not limited to, the following:

(i) the distinctive designation of such series and the number of shares which shall constitute such series, which number may be increased (except where otherwise provided by the Board of Directors in creating such series) or decreased (but not below the number of shares thereof then outstanding) from time to time by like action of the Board of Directors;

(ii) the annual rate of dividends payable on shares of such series, the conditions upon which such dividends shall be payable and the date from which dividends shall be cumulative in the event the Board of Directors determines that dividends shall be cumulative;

(iii) whether such series shall have voting rights, in addition to the voting rights provided by law and, if so, the terms of such voting rights;

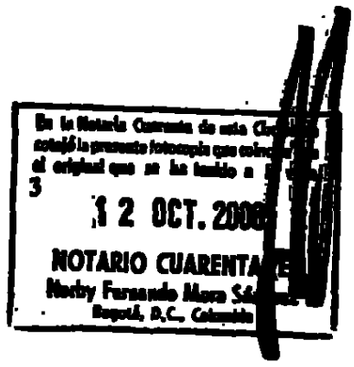
(iv) whether such series shall have conversion privileges and, if so, the terms and conditions of such conversion, including, but not limited to, provision for adjustment of the conversion rate upon such events and in such manner as the Board of Directors shall determine;

(v) whether or not the shares of such series shall be redeemable and, if so, the terms and conditions of such redemption, including the date or dates upon or after which they shall be redeemable, and the amount per share payable in case of redemption, which amount may vary under different conditions and at different redemption dates;

(vi) whether such series shall have a sinking fund for the redemption or purchase of shares of that series and, if so, the terms and amount of such sinking fund;

(vii) the rights of the shares of such series in the event of voluntary or involuntary liquidation, dissolution or winding up of the Corporation, and the relative rights of priority, if any, of payment of shares of that series; and

(viii) any other relative rights, preferences and limitations of such series.



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ARTICLE 5

SECTION 5.01. *Amendment of Bylaws by Directors.* (a) In furtherance and not in limitation of the powers conferred by statute, the Board of Directors is expressly authorized to make, alter, amend, repeal and rescind the bylaws of the Corporation, subject to Sections 5.01(b) and 5.01(c) hereof.

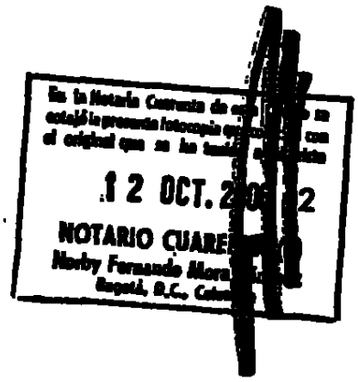
(b) Sections 2.02, 3.02, 3.03(a), 3.03(b), 3.04, 3.05, 3.14, and 5.06 of the bylaws may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 60% of the shares entitled to be voted thereon.

(c) Sections 3.03(c) and 3.15 of the bylaws may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 90% of the shares entitled to be voted thereon.

SECTION 5.02. *Amendment of Certificate of Incorporation.* Section 5.01(b) of this Certificate of Incorporation may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 60% of the shares entitled to be voted thereon. Section 5.01(c) and Articles 8 and 9 of this Certificate of Incorporation may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 90% of the shares entitled to be voted thereon.

ARTICLE 6

SECTION 6.01. *Election of Directors by Holders of Preferred Stock.* During any period when the holders of any Preferred Stock or any one or more series thereof, voting as a class, shall be entitled to elect a specified number of directors, by reason of dividend arrearages or other provisions giving them the right to do so, then and during such time as such right continues (i) the then otherwise authorized number of directors shall be increased by such specified number of directors, and the holders of such Preferred Stock or such series thereof, voting as a class, shall be entitled to elect the additional directors so provided for, pursuant to the provisions of such Preferred Stock or series; (ii) each such additional director shall serve for such term, and have such voting powers, as shall be stated in the provisions pertaining to such Preferred Stock or series; and (iii) whenever the holders of any such Preferred Stock or series thereof are



divested of such rights to elect a specified number of directors, voting as a class, pursuant to the provisions of such Preferred Stock or series, the terms of office of all directors elected by the holders of such Preferred Stock or series, voting as a class pursuant to such provisions or elected to fill any vacancies resulting from the death, resignation or removal of directors so elected by the holders of such Preferred Stock or series, shall forthwith terminate and the authorized number of directors shall be reduced accordingly.

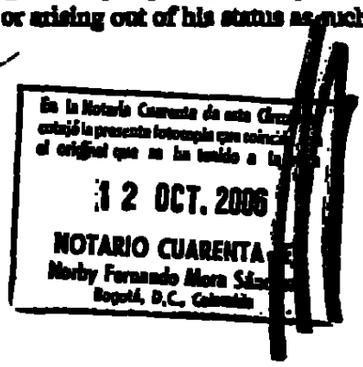
SECTION 6.02. *Ballots*. Elections of directors at an annual or special meeting of stockholders need not be by written ballot unless the bylaws of the Corporation shall provide otherwise.

SECTION 6.03. *Elimination of Certain Personal Liability of Directors*. (a) A director of the Corporation shall not be liable to the Corporation or its stockholders for monetary damages for breach of fiduciary duty as a director to the fullest extent permitted by Delaware Law.

(b) Each person (and the heirs, executors or administrators of such person) who was or is a party or is threatened to be made a party to, or is involved in any threatened, pending or completed action, suit or proceeding, whether civil, criminal, administrative or investigative, by reason of the fact that such person is or was a director of the Corporation or is or was serving at the request of the Corporation as a director of another corporation, partnership, joint venture, trust or other enterprise, shall be indemnified and held harmless by the Corporation to the fullest extent permitted by Delaware Law. The right to indemnification conferred in this Section 6.03 shall also include the right to be paid by the Corporation the expenses incurred in connection with any such proceeding in advance of its final disposition to the fullest extent authorized by Delaware Law. The right to indemnification conferred in this Section 6.03 shall be a contract right.

(c) The Corporation may, by action of its Board of Directors, provide indemnification to such of the officers, employees and agents of the Corporation to such extent and to such effect as the Board of Directors shall determine to be appropriate and authorized by Delaware Law.

(d) The Corporation shall have power to purchase and maintain insurance on behalf of any person who is or was a director, officer, employee or agent of the Corporation, or is or was serving at the request of the Corporation as a director, officer, employee or agent of another corporation, partnership, joint venture, trust or other enterprise against any expense, liability or loss incurred by such person in any such capacity or arising out of his status as such, whether or



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not the Corporation would have the power to indemnify him against such liability under Delaware Law.

(e) The rights and authority conferred in this Section 6.03 shall not be exclusive of any other right which any person may otherwise have or hereafter acquire.

(f) Neither the alteration, amendment, repeal or recission of this Section 6.03, nor the adoption of any provision of this Certificate of Incorporation or the bylaws of the Corporation, nor, to the fullest extent permitted by Delaware Law, any modification of law, shall eliminate or reduce the effect of this Section 6.03 in respect of any acts or omissions occurring prior to such alteration, amendment, repeal, recission, adoption or modification.

ARTICLE 7

SECTION 7.01. *Section 203 of Delaware Law.* The Corporation hereby elects not to be governed by Section 203 of Delaware Law.

ARTICLE 8

SECTION 8.01. *Relationship With Roche.* In anticipation that the Corporation will cease to be a wholly owned subsidiary of Roche Holdings, Inc., but that Roche (as defined in Section 8.05) will remain a stockholder of the Corporation, and in anticipation that the Corporation (as defined in Section 8.05) and Roche may engage in the same or similar activities or lines of business and have an interest in the same areas of corporate opportunities, and in recognition of (i) the benefits to be derived by the Corporation through its continued contractual, corporate and business relations with Roche (including service of officers and directors of Roche as directors of the Corporation) and (ii) the difficulties attendant to any director, who desires and endeavors fully to satisfy such director's fiduciary duties, in determining the full scope of such duties in any particular situation, the provisions of this Article 8 are set forth to regulate, define and guide the conduct of certain affairs of the Corporation as they may involve Roche and its officers and directors, and the powers, rights, duties and liabilities

of the Corporation and its officers, directors and stockholders in connection therewith.

SECTION 8.02. *Similar Business Activities.* Except as Roche Holdings, Inc. may otherwise agree in writing,

(a) Roche shall not have a duty to refrain from engaging directly or indirectly in the same or similar business activities or lines of business as the Corporation, and

(b) neither Roche nor any officer or director thereof shall be liable to the Corporation or its stockholders for breach of any fiduciary duty by reason of any such activities of Roche or of such person's participation therein.

In the event that Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for both Roche and the Corporation, Roche shall have no duty to communicate or offer such corporate opportunity to the Corporation and shall not be liable to the Corporation or its stockholders for breach of any fiduciary duty as a stockholder of the Corporation or controlling person of a stockholder by reason of the fact that Roche pursues or acquires such corporate opportunity for itself, directs such corporate opportunity to another person or entity, or does not communicate information regarding, or offer, such corporate opportunity to the Corporation.

SECTION 8.03. *Corporate Opportunities.* In the event that a director, officer or employee of the Corporation who is also a director, officer or employee of Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for the Corporation and Roche (whether such potential transaction or matter is proposed by a third-party or is conceived of by such director, officer or employee of the Corporation), such director, officer or employee shall be entitled to offer such corporate opportunity to the Corporation or Roche as such director, officer or employee deems appropriate under the circumstances in his sole discretion, and no such director, officer or employee shall be liable to the Corporation or its stockholders for breach of any fiduciary duty or duty of loyalty or failure to act in (or not opposed to) the best interests of the Corporation or the derivation of any improper personal benefit by reason of the fact that (i) such director, officer or employee offered such corporate opportunity to Roche (rather than the Corporation) or did not communicate information regarding such corporate opportunity to the Corporation or (ii) Roche pursues or acquires such corporate opportunity for itself or directs such corporate opportunity to another person or does not communicate information regarding such corporate opportunity to the Corporation.

SECTION 8.04. *Deemed Consent of Stockholders.* Any person or entity purchasing or otherwise acquiring any interest in any shares of capital stock of the Corporation shall be deemed to have notice of and to have consented to the provisions of this Article 8.

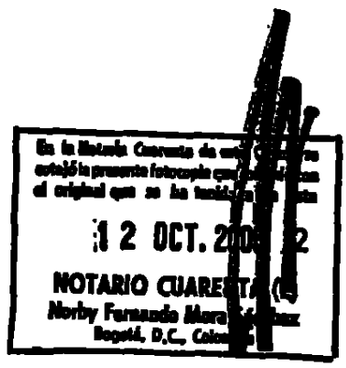
SECTION 8.05. *Definitions.* For purposes of this Article 8 and Article 9 only, (i) the term "Corporation" shall mean the Corporation and all corporations, partnerships, joint ventures, associations and other entities in which the Corporation beneficially owns (directly or indirectly) fifty percent or more of the outstanding voting stock, voting power or similar voting interests, and (ii) the term "Roche" shall mean Roche Holdings, Inc. and all corporations, partnerships, joint ventures, associations and other entities (other than the Corporation, defined in accordance with clause (i) of this Section 8.05) that are "affiliates" (as defined in Rule 12b-2 under the Securities Exchange Act of 1934, as amended) of Roche Holdings, Inc.

SECTION 8.06. *Termination: Binding Effect.* Notwithstanding anything in this Certificate of Incorporation to the contrary, the foregoing provisions of this Article 8 shall expire on the date that Roche ceases to own beneficially Common Stock representing at least 5% of the number of outstanding shares of Common Stock of the Corporation and no person who is a director or officer of the Corporation is also a director or officer of Roche. Neither such expiration, nor the alteration, amendment, change or repeal of any provision of this Article 8 nor the adoption of any provision of this Amended and Restated Certificate of Incorporation inconsistent with any provision of this Article 8 shall eliminate or reduce the effect of this Article 8 in respect of any matter occurring, or any cause of action, suit or claim that, but for this Article 8, would accrue or arise, prior to such expiration, alteration, amendment, repeal or adoption.

SECTION 8.07. *Article 9.* The provisions of this Article 8 are in addition to the provisions of Article 9.

ARTICLE 9

SECTION 9.01. *Contracts Not Void.* No contract, agreement, arrangement or transaction (or any amendment, modification or termination thereof) between the Corporation and Roche shall be void or voidable solely for the reason that Roche is a party thereto, or solely because any directors or officers of the Corporation who are affiliated with Roche are present at or participate in the



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meeting of the Board of Directors or committee thereof which authorizes the contract, agreement, arrangement, transaction, amendment, modification or termination or solely because his or their votes are counted for such purpose, but any such contract, agreement, arrangement or transaction (or any amendment, modification or termination thereof) shall be governed by the provisions of this Amended and Restated Certificate of Incorporation, the Corporation's Bylaws, Delaware Law and other applicable law. For purposes of this Article 9, the terms the "Corporation" and "Roche" have the meanings set forth in Section 8.05.

SECTION 9.02. *Quorum.* Directors of the Corporation who are also directors or officers of Roche may be counted in determining the presence of a quorum at a meeting of the Board of Directors or of a committee that authorizes or approves any such contract, agreement, arrangement or transaction (or amendment, modification or termination thereof). Outstanding shares of Common Stock owned by Roche may be counted in determining the presence of a quorum at a meeting of stockholders that authorizes or approves any such contract, agreement, arrangement or transaction (or amendment, modification or termination thereof).

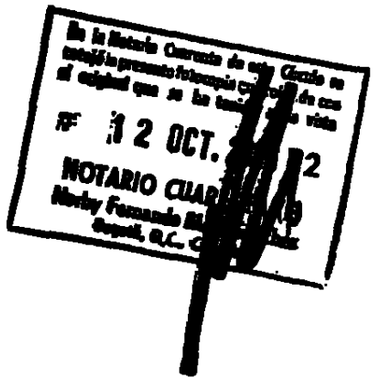
SECTION 9.03. *No Liability For Good Faith Actions.* Neither Roche nor any officer or director thereof shall be liable to the Corporation or its stockholders for breach of any fiduciary duty or duty of loyalty or failure to act in (or not opposed to) the best interests of the Corporation or the derivation of any improper personal benefit by reason of the fact that Roche or an officer of director thereof in good faith takes any action or exercises any rights or gives or withholds any consent in connection with any agreement or contract between Roche and the Corporation. No vote cast or other action taken by any person who is an officer, director or other representative of Roche, which vote is cast or action is taken by such person in his capacity as a director of this Corporation, shall constitute an action of or the exercise of a right by or a consent of Roche for the purpose of any such agreement or contract.

SECTION 9.04. *Deemed Consent by Stockholders.* Any person or entity purchasing or otherwise acquiring any interest in any shares of capital stock of the Corporation shall be deemed to have notice of and to have consented to the provisions of this Article 9.

SECTION 9.05. *Contracts Covered.* For purposes of this Article 9, any contract, agreement, arrangement or transaction with the Corporation or any corporation, partnership, joint venture, association or other entity in which the Corporation beneficially owns (directly or indirectly) fifty percent or more of the outstanding voting stock, voting power or similar voting interests shall be deemed to be a contract, agreement, arrangement or transaction with the Corporation.

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

APOSTILLA

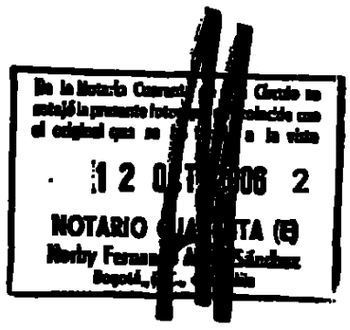
(Convención de La Haya del 5 de Octubre de 1961)

1. País: Estados Unidos de América
   Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

Certificado

5. en Dover, Delaware
6. el veinticuatro de mayo, A. D. 2005
7. por el Secretario de Estado, Departamento de Estado de Delaware
8. No. 0254030
9. Sello/timbre 10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado



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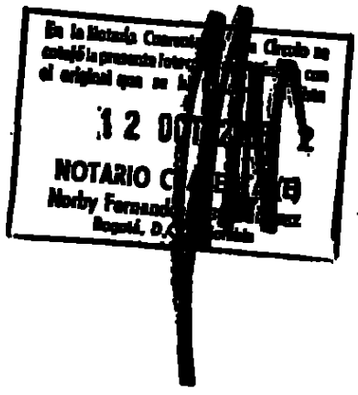
DELAWARE

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.



Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900384

FECHA: 05 – 24 – 05

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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 ser modificada la misma en adelante ("**Delaware Law**").

ARTICULO 4

SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de cuatrocientos millones (400,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones

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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.

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particulares de las acciones de tal serie (siempre y cuando no sean inconsistentes con las condiciones de este Artículo 4 aplicables a todas las series de Acciones Preferenciales), incluyendo, pero sin limitarse a, las siguientes:

(i) la designación distintiva de tal serie y el número de las acciones que han de constituir tal serie, numero que puede incrementarse (excepto cuando lo determine de otro modo la Junta Directiva al crear tal serie) o reducida (pero no por debajo del número de acciones de las mismas que están vigentes) cada cierto tiempo por una acción semejante de la Junta Directiva;

(ii) la tasa anual de dividendos a pagar sobre las acciones de tal serie, las condiciones sobre las cuales se pagarán tales dividendos y le fecha a partir de la cual los dividendos serán acumulativos en el evento que la Junta Directiva determine que los dividendos serán acumulativos;

(iii) si la serie tendrá o no derechos de voto, además de los derechos de voto provistos por la ley, y de ser así, los términos de tales derechos de voto;

(iv) si tal serie tendrá o no privilegios de conversión y, de ser así, los términos y condiciones de tal conversión, incluyendo, pero sin estar limitado a, la condición para ajuste de la tasa de conversión en tales eventos y de tal forma como la Junta Directiva lo determine;

(v) si las acciones de tal serie serán o no redimibles y, de ser así, los términos y condiciones de tal redención, incluyendo la fecha o fechas en las cuales o después de las cuales pueden ser redimibles, y la cantidad a pagar por acción en caso de redención, cantidad que variará bajo diferentes condiciones y en diferentes fechas de redención;

(vi) si tal serie tendrá o no un fondo de inversión para la redención o compra de acciones de esa serie y, de ser así, los términos y la cantidad de tal fondo de inversión;

(vii) los derechos de las acciones de tal serien caso de una liquidación, disolución o cierre voluntaria o involuntaria de la Corporación, y

los derechos relativos de prioridad, de haber, de pago de acciones de esa serie; y

(viii) cualquier otro derecho relativo, preferencia y limitación de tal serie.

ARTICULO 5

SECCION 5.01. *Enmiendas a los estatutos por los Directores.* (a) En adición y no limitando los poderes conferidos por estatutos, la Junta Directiva esta expresamente autorizada para hacer, alterar, enmendar, rechazar y rescindir los estatutos de la Corporación, sujetos a las secciones 5.01(b) y 5.01(c) a continuación.

(b) Las secciones 2.02, 3.02, 3.03(a), 3.04, 3.05, 3.14 y 5.06 de los estatutos solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 60% de las acciones con derecho a votar las mismas.

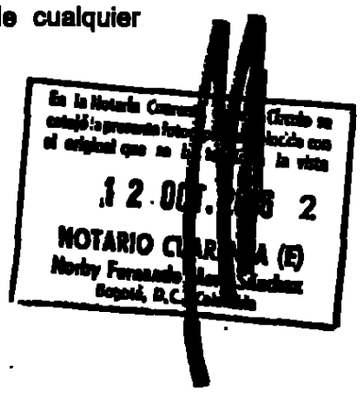
(c) Las secciones 3.03(c) y 3.15 de los estatutos solo pueden ser alteradas, enmendadas, repelidas, o rescindidas por le voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 90% de las acciones con derecho a votar las mismas.

o

SECCION 5.02. *Enmienda del Certificado de Incorporación.* La sección 5.01 (b) de este Certificado de Incorporación solo puede ser alterada, enmendada, repelida, o rescindida por el voto afirmativo de los accionistas de capital con derecho a voto sobre la misma que representen a más del 60% de las acciones con derecho a votar la misma. La sección 5.01(c) y los Artículos 8 y 9 de este Certificado de Incorporación solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar la misma que representen más del 90% de las acciones con derecho a votar la misma.

ARTICULO 6

SECCION 6.01. *Elección del Directorio por los Accionistas de Acción Preferencial.* Durante cualquier periodo cuando los accionistas de cualquier



acción preferencial cualquiera de una o más series de las mismas, que voten como una clase, tengan derecho a elegir un número específico de directores, por razón a atrasos en los dividendos u otras condiciones que les dan el derecho a hacerlo, entonces y durante el tiempo que continúe el derecho (i) el número de directores autorizado de otro modo será incrementado por tal número de directores especificado, y los accionistas de tales Acciones Preferenciales o tales series de las mismas, que votan como una clase, tendrán derecho a elegir los directores adicionales para lo que se provee, de acuerdo con las condiciones de tales Acciones Preferenciales o series; (ii) cada uno de tales directores adicionales servirá durante tal termino, y tendrá tales poderes de voto, como se han de otorgar en las provisiones pertinentes a tales Acciones Preferenciales o series; y (iii) siempre que los accionistas de cualquiera de tales Acciones Preferenciales o series de las mismas están desprovistos de tales derechos a elegir un número específico de directores, votando como clase, de acuerdo con las condiciones de tales Acciones preferenciales o series, los términos de oficio de todos los directores elegidos por los accionistas de tales Acciones preferenciales o series, que votan como clase, de acuerdo con las condiciones o elegidos para llenar cualquier vacante resultante de la muerte, renuncia o remoción de directores así elegidos por los accionistas de tales Acciones Preferenciales o series, terminarán en adelante y el número autorizado de directores será reducido en concordancia.

SECCION 6.02. *Votaciones.* Las elecciones de directores en una reunión anual o especial de accionistas necesaria no serán por votos escritos a menos que los estatutos de la Corporación digan lo contrario.

SECCION 6.03. *Eliminación de cierta responsabilidad personal de los directores.* (a) Un director de la Corporación no será responsable ante la Corporación o sus accionistas por daños monetarios por violación del deber fiduciario como director hasta dondé lo permita la Ley de Delaware.

(b) Cada persona (y sus herederos, ejecutores o administradores de tal persona) quien fue o es una parte o esta amenazado de ser parte de, o esta involucrado en cualquier acción, demanda o proceso amenazado, pendiente, o completado, ya sea civil, criminal, administrativo o investigativo, por razón del hecho de que esa persona es o fue un director de la Corporación o es o estaba sirviendo por solicitud de la Corporación como un director de otra corporación, sociedad, sociedad de riesgo compartido, fideicomiso u otra empresa, será indemnizado y completamente protegido por la Corporación hasta donde lo

permita la Ley de Delaware. El derecho a la indemnización concedido en esta sección 6.03 también debe incluir el derecho a que la Corporación le pague los gastos incurridos en conexión cuna cualquiera de tales procesos por adelantado de su decisión final hasta donde los autoriza la Ley de Delaware. El derecho a indemnización conferido en esta sección 6.03 será un derecho contractual.

(c) La Corporación puede, por acción de su Junta Directiva, proporcionar indemnización a tales funcionarios, empleados y agentes de la Corporación hasta tal punto y a tal efecto como lo determine apropiado la Junta Directiva y lo autorice la Ley de Delaware.

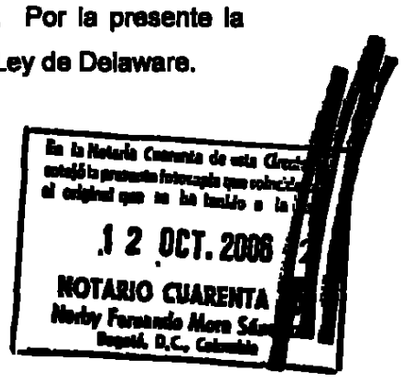
(d) La Corporación tendrá el poder de comprar y mantener un seguro a nombre de cualquier persona quien es o fue director, funcionario, empleado o agente de la corporación, o esta o estuvo en servicio por pedido de la Corporación como director, funcionario, empleado o agente de otra corporación, sociedad, sociedad de riesgo compartido, fideicomiso u otra empresa contra cualquier gasto, responsabilidad o pérdida incurrida por tal persona en cualquier capacidad o que surge de su estado como tal, ya sea si la Corporación tiene o no el poder de indemnizarlo contra tal responsabilidad bajo la ley de Delaware.

(e) Los derechos y autoridad conferidos en esta Sección 6.03 no serán exclusivos de ningún otro derecho el cual cualquier otra persona pueda tener o adquirir de aquí en adelante.

(f) Ni la alteración, enmienda, rechazo o rescisión de esta Sección 6.03, ni la adopción de ninguna condición de este Certificado de Incorporación o de los Estatutos de la Corporación, ni, hasta donde lo permite la Ley de Delaware, ninguna modificación de la ley, debe eliminar o reducir el efecto de esta Sección 6.03 respecto a ninguno de los actos u omisiones que ocurran antes de tal alteración, enmienda, rechazo, rescisión, adopción o modificación.

ARTICULO 7

SECCION 7.01, *Sección 203 de la Ley de Delaware*. Por la presente la Corporación elige no ser gobernada por la Sección 203 de la Ley de Delaware.



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ARTICULO 8

SECCION 8.01. *Relación con Roche.* Anticipándose a que la Corporación cese de ser un subsidiaria completamente de propiedad de Roche Holdings, Inc., pero que Roche (como se define en la sección 8.05) permanezca siendo un accionista de la Corporación, y anticipándose a que la Corporación (como se define en la Sección 8.05) y Roche puede entrar en actividades o líneas de negocios semejantes o similares y tener un interés en las mismas áreas de oportunidades corporativas, y en reconocimiento de (i) los beneficios a ser derivados por la Corporación a través de sus relaciones contractuales, corporativas y de negocios con Roche (incluyendo el servicio de funcionarios y directores de Roche como directores de la Corporación) y (ii) las dificultades concomitantes a cualquier director, quien desea y se propone satisfacer completamente tales deberes fiduciarios del director, al determinar el alcance completo de tales deberes en cualquier situación particular, las condiciones de este artículos 8 se establecen para regular, definir y guiar la conducta de ciertas cuestiones de la Corporación ya que pueden involucrar a Roche y sus funcionaros y directores, y los poderes, derechos, deberes y responsabilidades de la Corporación y sus funcionarios, directores y accionistas en conexión con éste.

SECCION 8.02. *Actividades en Negocios Similares.* Excepto que Roche Holdings, Inc. pueda de otra forma acordarlo por escrito,

(a) Roche no tendrá el deber de abstenerse de entrar directamente o indirectamente en actividades de negocios o líneas de negocios semejantes o similares a las de la Corporación, y

(b) ni Roche ni ninguno de sus funcionarios o director de la misma será responsable a la Corporación o sus accionistas por violar cualquier deber fiduciario por razón de alguna de tales actividades de Roche o de la participación de tal persona en las mismas.

En el evento que Roche adquiera conocimientos de una transacción o materia potencial que pueda ser una oportunidad corporativa tanto para Roche como para la Corporación, Roche no tendrá el deber de comunicar u ofrecer tal oportunidad corporativa a la Corporación y no será responsable ante la Corporación o sus accionistas por violar ningún deber fiduciario como accionista de la Corporación o la persona que controla a un accionista por razón del hach

que Roche prosigue o adquiere tal oportunidad corporativa para si misma, dirige tal oportunidad corporativa a otra persona o entidad, o no comunica información respecto, u ofrece, tal oportunidad corporativa a la Corporación.

SECCION 8.03. *Oportunidades Corporativas.* En el evento que un director, funcionario o empleado de la Corporación quien también es director, funcionario o empleado de Roche adquiera conocimiento de una transacción o materia potencial que pueda ser una oportunidad corporativa para la Corporación y Roche (ya se si esta transacción o materia potencial es propuesta por un tercero o es concebida por tal director, funcionario o empleado de la Corporación o no), tal director, funcionario o empleado debe tener el derecho a ofrecer tal oportunidad corporativa a la Corporación o Roche como tal director, funcionario o empleado los considere apropiado bajo las circunstancias de su discreción, y tal director, funcionario o empleado no será responsable a la Corporación o sus accionistas por violar ningún deber fiduciario o deber de lealtad o faltar en actuar en (o no oponerse a) los mejores intereses de la Corporación o la derivación de ningún beneficio personal inapropiado por razón del hecho que (i) tal director, funcionario o empleado ofreció tal oportunidad corporativa a Roche (en lugar de a la Corporación) o no comunicó información respecto a tal oportunidad corporativa a la Corporación o (ii) Roche busca o adquiere tal oportunidad corporativa para si misma o dirige tal oportunidad corporativa a otra persona o no comunica información respecto a tal oportunidad corporativa a la Corporación.

SECCION 8.04. *Consentimiento estimado de los accionistas.* Se considerará que cualquier persona o entidad que compre o de otro modo adquiera algún interés en cualquiera de las acciones de capital de la Corporación ha tenido noticia o ha estado de acuerdo con las condiciones de este Artículo 8.

SECCION 8.05, *Definiciones.* Para propósitos de este Artículo 8 y el Artículo 9 solamente, (i) el término ***"Corporación"*** debe significar la Corporación y todas las corporaciones, sociedades, sociedades de riesgo compartido y otras entidades en las cuales la Corporación posee con beneficio (directa o indirectamente) cincuenta por ciento o más de las acciones con voto, poder de voto o intereses de voto similares, y (ii) el término ***"Roche"**** debe significar Roche Holdings, Inc y todas las corporaciones, sociedades, sociedades de riesgo compartido, asociaciones y otras entidades (diferentes a la Corporación, definida de acuerdo con la cláusula (I) de esta sección 8.05) que son ***"afiliados"*** (como se

define en la Regla 12b-2 bajo el Acta de Intercambio de Valores de 1934, como se enmendó) de Roche Holdings, Inc.

SECCION 8.06, *Finalización: Efecto Obligante.* Son consideración con nada en este Certificado de Incorporación que lo contradiga, las condiciones anteriores de este Artículo 8 deberán expirar en la fecha que Roche cese de poseer con beneficios Acciones Comunes que representen al menos 5% del número de acciones vigentes de las Acciones Comunes de la Corporación y nadie que se director o funcionario de la Corporación sea también director o funcionario de Roche. Ni tal vencimiento, ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 8 así como tampoco ninguna condición de este Certificado de Incorporación Enmendado y Vuelto a Redactar inconsistente con alguna de las condiciones de este Artículo 8 eliminará o reducirá el efecto de este Artículo 8 respecto a cualquier materia, o cualquier causa de acción, demanda o reclamo que, excepto por este Artículo 8, pueda acaecer o surgir, antes de tal vencimiento, alteración, enmienda o adopción.

SECCION 8.07. *Artículo 9.* Las condiciones de este Artículo son adicionales a las condiciones del Artículo 9.

ARTICULO 9

SECCION 9.01. *Contratos no nulos.* Ningún contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización de los mismos) entre la Corporación y Roche será nulo u anulable solamente por razón de que Roche es parte del mismo, o solamente porque cualquiera de los directores o funcionarios de la Corporación quienes están afiliados a Roche están presentes en o participan en la reunión de la Junta Directiva o comité de la misma que autoriza el contrato, acuerdo, arreglo, transacción, enmienda, modificación o finalización o solamente porque su o sus votos se cuentan para tal propósito, pero cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo) estará gobernado por las condiciones de este Cerificado de Incorporación Enmendado y vuelto a Redactar, los Estatutos de la Corporación, la Ley de Delaware y otras leyes aplicables. Para propósitos de este Artículo 9, los términos "***Corporación***" y "***Roche***" tienen los significados establecidos en la Sección 8.05.

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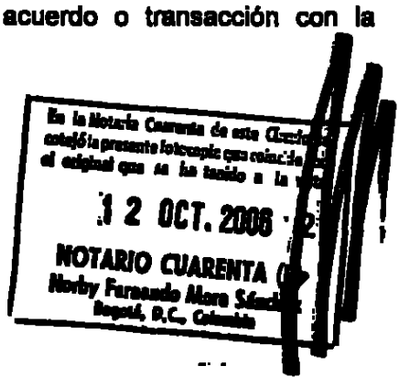
SECCION 9.02. *Quórum.* Los Directores de la Corporación que son también directores o funcionarios de Roche pueden contarse al determinar la presencia de un quórum en una reunión de la Junta Directiva o de un comité que autoriza o aprueba cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo). Las acciones vigentes de las Acciones Comunes de propiedad de Roche pueden contarse al determinar la presencia de un quórum en una reunión de accionistas que autorice o apruebe tal contrato, acuerdo, arreglo o transacción (o cualquier enmienda, modificación o finalización del mismo).

SECCION 9.03. *No responsabilidad por acciones de buena fe.* Ni Roche ni ningún funcionario o director de la misma será responsable ante la Corporación o sus accionistas por violar cualquiera de los deberes fiduciarios o deberes de lealtad o por fallar en actuar en (o no oponerse a) los mejores intereses de la Corporación o por derivación de cualquier beneficio personal por razón del hecho que Roche o un funcionario o director de la misma de buena fe tome cualquier acción o ejerza cualquier derecho o de o retenga cualquier consentimiento en conexión con cualquier acuerdo o contrato entre Roche y la Corporación. Ningún voto dado u otra acción tomada por cualquier persona que es un funcionario, director o representante de Roche, donde el voto o acción es tomado por tal persona en su capacidad como director de esta Corporación, constituirá una acción de o el ejercicio de un derecho o un consentimiento de Roche para los propósitos de cualquiera de tal acuerdo o contrato.

SECCION 9.04. *Consentimiento estimado de los accionistas.* Se considerará que cualquier persona o entidad que compre o de otro modo adquiera algún interés en cualquiera de las acciones de capital de la Corporación ha tenido noticia o ha estado de acuerdo con las condiciones de este Artículo 9.

SECCION 9.05. *Contratos cubiertos.* Para propósitos de este Artículo 9, cualquier contrato, acuerdo, arreglo o transacción con la Corporación o cualquier corporación, sociedad, sociedad de riesgo conjunto, asociación u otra entidad en la cual la Corporación posee con beneficio (directa o indirectamente) el cincuenta por ciento o más de las acciones con voto, poderes de voto o intereses de voto similares vigentes se considerará un contrato, acuerdo o transacción con la Corporación.



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SECCION 9.06. *Efecto obligante.* Ni la alteración, enmienda, cambio o rechazo de alguna de las condiciones de este Artículo 9 ni la adopción de alguna condición inconsistente con cualquier condición de este Artículo 9 eliminará o reducirá el efecto de este Artículo 9 respecto a cualquier materia que ocurra, o cualquier causa de acción, demanda o reclamo que, a no ser por este Artículo 9, pueda acaecer o surgir, antes de tal alteración, enmienda, cambio, rechazo o adopción.

SECCION 9.07. *Artículo 8.* Las condiciones de este Artículo 9 son adicionales a las condiciones del Artículo 8.

EN TESTIMONIO DE LO CUAL, este Certificado de Incorporación Enmendado y vuelto a Redactar, el cual ha sido adoptado de acuerdo con las secciones 242 y 245 de la Ley General de Corporación del Estado de Delaware y por consentimiento escrito de el único accionista de la Corporación de acuerdo con la sección 228 de Ley General de Corporación del Estado de Delaware, ha sido ejecutado este 22 de Julio de 1999.

GENENTECH, INC.
Por: /s/ Stephen G. Juelsgaard
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente
Consejo General y Secretaría

En la Notaría Cuarenta de esta Ciudad
extajó la presente fotocopia que coincide
del original que se le tuvido a la
12 OCT. 2006
NOTARIO CUARENTA
Rocky Fernando Mora Sá
Bogotá, D.C., Colombia

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3 F 8

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

De la Notaría Cuarenta de este Círculo
contiguo la presente fotocopia que coincide con
el original que se ha tenido a la vista

12 OCT. 2006

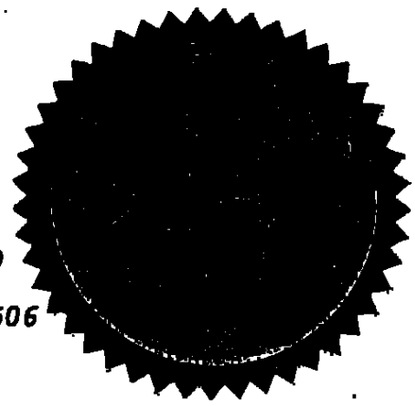
NOTARIO CUARENTA (
Nieby Fernando Mora Sánchez
Bogotá, D.C., Colombia

Delaware

409
349

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE FIFTEENTH DAY OF MAY, A.D. 2000, AT 5 O'CLOCK P.M.



Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900385

DATE: 05-24-05

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 05:00 PM 05/15/2000
001245251 - 2112540

G/D 350

CERTIFICATE OF AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> "Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is seven hundred million (700,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Six hundred million (600,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 15th day of May, 2000.

GENENTECH, INC.

By: _____
Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel and Secretary

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En la Notaría Cuarenta de esta Ciudad se extrajó la presente fotocopia que coincide con el original que se ha tenido a la vista.
12 OCT. 2000
NOTARIO CUARENTA (E)
Norby Fernando Mora
Bogotá, D.C., Colombia

351

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

352

412

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO VUELTO A REDACTAR DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMOQUINTO DIA DE MAYO, A. D. 2000, A LAS 5 EN PUNTO DE LA TARDE.

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900385

FECHA: 05 – 24 – 05

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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.

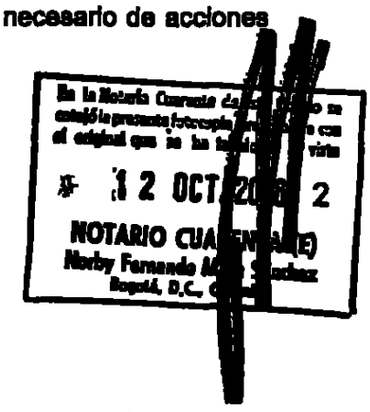
CERTIFICA POR LA PRESENTE:

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 y se sostuvo al ser anunciada de acuerdo con la sección 222 de la Ley General de Corporación del Estado de Delaware, en donde el número necesario de acciones requerido por los estatutos votaron a favor de la enmienda.



TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 15 de Mayo de 2000.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.

(41) 355

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

12 OCT. 2006

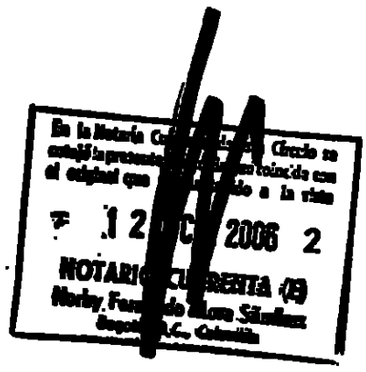
NOTARIO CUARENTA

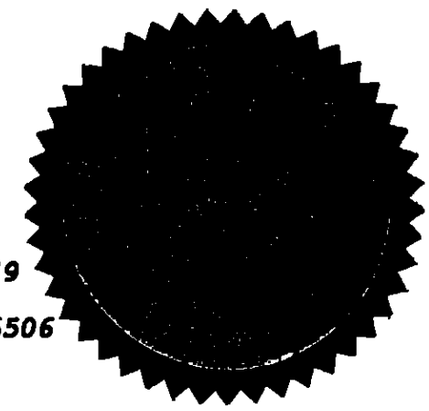
Delaware

47-6
356

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE SIXTEENTH DAY OF APRIL, A.D. 2004, AT 7:39 O'CLOCK P.M.





Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

2112549

050426506

AUTHENTICATION: 3900387

DATE: 05-24-05

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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 - 2112549 FILE

CERTIFICATE OF THIRD AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

Genentech, Inc., a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware,

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

> Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is three billion one hundred million (3,100,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value $0.02 per share ("Preferred Stock"). Three billion (3,000,000,000) shares shall be designated common stock, par value $0.02 per share ("Common Stock"). The common stock of the Corporation shall be all of one class.

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Third Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 16th day of April, 2004.

GENENTECH, INC.

By: _____

Name: Stephen G. Juelsgaard

Title: Executive Vice President, General Counsel and Secretary

#153087

En la Notaría Cuarenta de [illegible] cotejó la presente fotocopia con el original que se [illegible]

12 OCT. 2004

NOTARIO CUARENTA (E)

Norby Fernando Mon[illegible]ez

Bogotá, D.C., Colombia

HTV
358

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. 0254033
9. Sello/timbre 10. Firma

Harriet Smith Windsor (Firma)
Secretaria de Estado

8119
359

DELAWARE

EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO DE ENMIENDA DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMOSEXTO DIA DE ABRIL, A. D. 2004, A LAS 7:39 EN PUNTO DE LA TARDE.

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900387

FECHA: 05 – 24 – 05

CERTIFICADO DE TERCERA ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

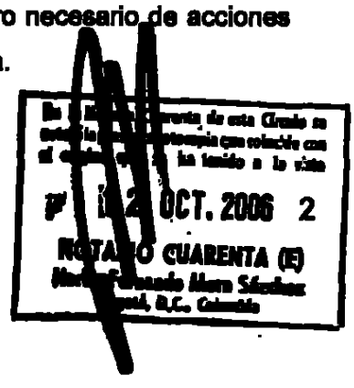
CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de tres mil cien millones (3,100,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Tres mil millónes (3,000,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de su Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente y se sostuvo al ser anunciada de acuerdo con la sección 222 de la Ley General de Corporación del Estado de Delaware, en donde el número necesario de acciones requerido por los estatutos votaron a favor de la enmienda.



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361

TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 16 de Abril de 2004.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America

This public document:

2. has been signed by Harriet Smith Windsor

3. acting in the capacity of Secretary of State of Delaware

4. bears the seal/stamp of Office of Secretary of State

Certified

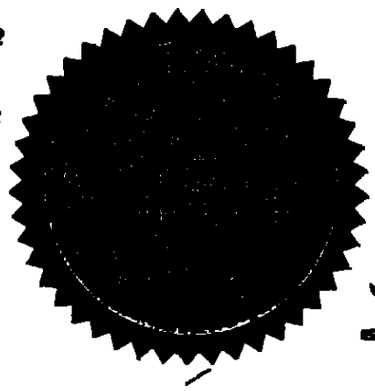
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254032

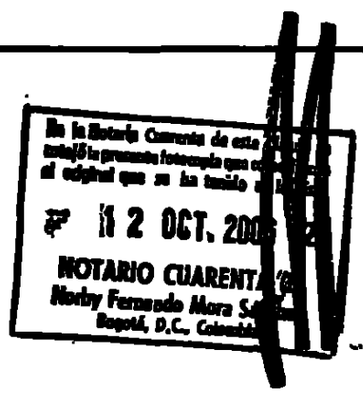
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



Delaware

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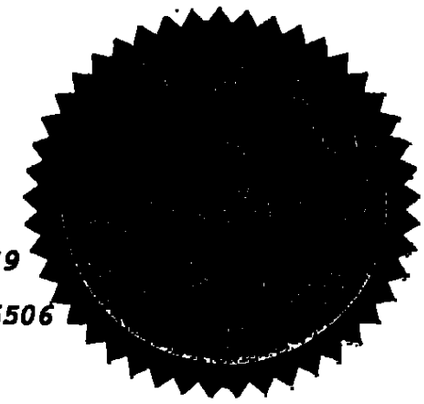
467

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: 05-24-05

STATE OF DELAWARE
SECRETARY OF STATE
DIVISION OF CORPORATIONS
FILED 02:05 PM 05/10/2001
010226224 – 2112549

S ////STATE/OF/DELAWARE///// V
T ///SECRETARY/OF/STATE///// O
A DIVISION/OF/CORPORATIONS// I
M FILED/02/05/PM/05/10/2001/ D
P ///010226224/-/2112549////
BY Sandy Miller

CERTIFICATE OF AMENDMENT OF AMENDED AND RESTATED CERTIFICATE OF INCORPORATION

364 424

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 G. Juelsgaard
Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel and Secretary

86747

En la Notaría Cuarenta de este Círculo se protocolizó la presente fotocopia que coincide con el original que se ha tenido a la vista
12 OCT. 2006
NOTARIO CUARENTA
Harby Fernando Mora Sánchez
Bogotá, D.C., Colombia

TRADUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

47
365

APOSTILLA

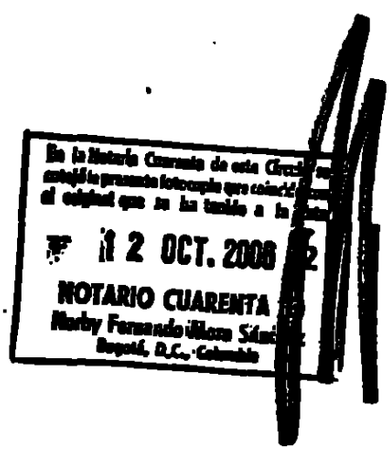
(Convención de La Haya del 5 de Octubre de 1961)

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



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366

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.

De la Notaría Cuarenta de este Círculo se extrajo la presente fotocopia que coincide con el original que se ha tenido a la vista

12 OCT. 2006

NOTARIO CUARENTA (E)
Harley Fernando Mora Sánchez
Bogotá, D.C., Colombia

Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900386

FECHA: 05 – 24 – 05

427
367

CERTIFICADO DE TERCERA ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

> SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de mil trescientos millones (1,300,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de $0.02 por acción ("**Acción Preferencial**"). Mil doscientos millones (1,200,000,000) de acciones serán designadas acciones comunes, por un valor de $0.02 por acción ("**Acción Común**"). La acción común de la Corporación será toda de una misma clase.

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de su Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente y se sostuvo al ser anunciada de acuerdo con la sección 222 de la Ley General de Corporación del Estado de Delaware, en donde el número necesario de acciones requerido por los estatutos votaron a favor de la enmienda.

[illegible] 12 OCT. 2006 2
NOTARIO CU[illegible] (E)
Nicky Fernando [illegible]
Bogotá, D.C., [illegible]

TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 10 de Mayo de 2001.

GENENTECH, INC.

Por: (Firma)
Nombre: Stephen G. Juelsgaard
Título: Vicepresidente, Consejo
y Secretaría General.

369

PETITORIO

AS: 102.493

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-121070- -00000-0000
Fec: 2006-11-30 16:10:41 Dep: 2020 NUECREACIONES
Tra: 11 PATENTEIYII
Cla: 378 FASENACIONALI
Act 411 PRESENTACION Folios: 126

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL

04/01/07

1 SOLICITUD DE:

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

☒ Capítulo I. ☐ Capítulo II.

2 SOLICITANTE (71)	Nombre: GENENTECH, INC. Dirección: 1 DNA Way, South San Francisco, CA 94080-4990, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail	**IDENTIFICACION** C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número ____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Carrera 7 # 71 – 21 Torre B – Piso 4 Teléfono: 744-2200 Fax: 744-2700 E-mail:	**IDENTIFICACION** C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual____ Número 80.410.337 TP 57492
4 INVENTOR (ES) (72)	Nombre: Paul A. Frohna Dirección: 3880 19th Street, San Francisco, CA 94114, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos de América	

5 PCT (86)
Solicitud Internacional No. PCT/US2005/019641 Fecha 02-06-2005
Publicación Internacional No. WO 2005/117978 A3 Fecha 15-12-2005

6 Título (54): MÉTODO PARA TRATAR LA ESCLEROSIS MÚLTIPLE

7 Clasificación Internacional (51) ____

8 Prioridad SI ☒ NO ☐	(33) País de Origen	(32) Fecha	(31) Número de Solicitud
	US	04-06-2004	60/576,993

9. Comprobante de pago No. 06-76,646 **Fecha** 30-11-2006

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

2020-F06 (01-11-15)

AP 370

REIVINDICACIONES

QUE SE REIVINDICA ES:

1. Un método para tratar esclerosis múltiple en un sujeto que comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto para suministrar una exposición inicial a anticuerpo de aproximadamente 0.5 a 4 gramos seguido por una segunda exposición de anticuerpo de aproximadamente 0.5 a 4 gramos, la segunda exposición no se suministra hasta aproximadamente 16 a 60 semanas a partir de la exposición inicial, y cada una de las exposiciones de anticuerpo se suministra al sujeto como una o dos dosis de anticuerpo.

2. El método de la reivindicación 1 en donde la segunda exposición no se suministra hasta de aproximadamente 20 a 30 semanas partir de la exposición inicial.

3. El método de la reivindicación 1 o 2 en donde la exposición inicial y la segunda exposición de anticuerpo cada una se suministran en cantidades de aproximadamente 1.5 a 2.5 gramos.

4. El método de una cualquiera de las reivindicación 2-3 que comprende adicionalmente administrar al sujeto una cantidad efectiva del anticuerpo CD20 para suministrar una tercera

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Rad: 06-121070- -00000-0000
Fec: 2006-11-30 16:10:41 Dep: 2020 NUECREACIONES
Tra: 11 PATENTE(VI)
Cue: 378 FASENACIONAL
Act: 411 PRESENTACION Folios: 428

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 [NO]
- Comprobante de pago de las tasas establecidas [X]

COMPLETA [ ] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Firma Responsable

Fecha Nov 30 de 2006

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

372

Bogotá,
2020

Doctor(a)
CADENA SARMIENTO JUAN PABLO
Apoderado(a) y/o Representante de
GENENTECH, INC.

REFERENCIA	Radicación No.	6 121070
	Solicitud internacional	PCT/US2005/019641
	Fecha inicio fase nacional	04/01/2007
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	0000 1879

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
lerazo 09 FEB. 2007